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*National Institute on Aging*  
*Report of program activities*  
NIA ANNUAL REPORT  
OCTOBER 1, 1977 THROUGH SEPTEMBER 30, 1978  
OFFICE OF THE DIRECTOR  
INFORMATION OFFICE

The NIA Information Office spent Fiscal Year 1978 increasing public awareness of Institute programs by publishing a variety of brochures and articles, providing summaries of selected NIA conferences, holding press conferences, preparing testimony, exhibiting at national meetings and conventions, and responding to letters and telephone inquiries.

The brochures published this year include:

- National Institute on Aging -- a description of the history, structure, programs, and goals of the new Institute.
- Changes: Research on Aging and the Aged -- a philosophical discussion of why research on aging is important, and how research advances can contribute to improvements in the quality of life for all Americans, especially the aged.
- To Understand the Aging Process: The Baltimore Longitudinal Study of Aging -- a detailed description of NIA's longitudinal study of normal aging from the perspective of a volunteer research subject.
- A Winter Hazard for the Old: Accidental Hypothermia -- a warning to the elderly to heat their homes to at least 65°F. to protect themselves from accidental hypothermia, a progressive drop in deep body temperature that can be fatal if it is not detected in time and treated properly.
- Special Report on Aging: 1978 -- a summary of NIA research advances during 1977 and the early part of 1978.
- Alternatives to Retirement, Energy and Aging, Nutrition and Aging -- Congressional testimony on these subjects, reproduced in large type so that those elderly with visual impairments will be able to read these statements.
- Report of the Panel on Behavioral and Social Sciences Research, Report of the Panel on Biomedical Research, Report of the Panel on Human Services and Delivery Systems, Summary Reports -- the detailed findings of the three panels which were formed to prepare "Our Future Selves," the DHEW-wide research plan on aging.
- Aging: Genetics and the Environment, Diabetes and Aging, Drugs and the Elderly, Is Mandatory Retirement Necessary?, Nutrition and Aging, Patterns of Disease Among the Aged, The Normality of Aging: The Baltimore Longitudinal Study, Thoughts on Geriatric Medicine, Using Biofeedback with the Elderly -- summaries of presentations given at a State of the Art Seminar on Aging Research.

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- NIA Cultured Cell Repository -- a description of a current contract with the Institute for Medical Research in Camden, N.J. to establish, characterize, store, and distribute standard and genetically-marked human cell lines for research on aging.
- Welcome to the NIA -- a personnel guide and maps for visitors to the NIA on the NIH campus and at the Gerontology Research Center in Baltimore.

The Information Office also cooperated with the Director in the preparation of the following articles, which appeared in a variety of journals and books:

- "Senility," "Nutrition," and "Accidental Hypothermia" for use in NIH: The Search for Health (1977)
- "The Older American Traveler" and "The Need for Teaching Geriatric Medicine" for the International Journal of Aging and Human Development
- "Thoughts on Aging" for the American Journal of Psychiatry
- Letters to the Editor: "To Remove a Dying Patient's Pain" (New York Times); "Sharing the Social Security Dollar" (Washington Post); "Helping the Older Mind" (Washington Star)
- "A Consummation Devoutly to be Wish'd" for Medical World News
- "The Older Physician" for the American Retired Physicians Association newsletter
- "The Doctor-Aged Patient Relationship" for Hospital Practice
- "A Winter Hazard for the Old: Accidental Hypothermia" for Nursing Care
- "Old Age" and "National Institute on Aging" for Encyclopedia Americana
- "Humanistic Perspectives in Gerontology" for the book Aging and the Elderly
- "Aging Research in the 20th Century" for book in honor of Professor Chebotarev
- "Senility" for book by Ann Landers
- "Senility" for World Book Encyclopedia



- "Geriatric Medicine, Geriatric Nursing, and Medical Gerontology" for the third annual meeting of Directors of National Institutes with Programs in the Field of Aging
- "Caring for the Elderly" for the Journal of Gerontological Nursing
- "Just Old Age?" for New Age
- "Crises of Aging and Alcohol" for Fairfax County
- Foreward to Aging: A Complex Management
- Introductory Statement for "Medication Management and Education of the Elderly," an Excerpta Medica Symposium
- Editorial for Psychosomatic Medicine
- "Changing Education and New Research in Geriatric Medicine" for the Physicians and Surgeons Journal
- "Alzheimer's Disease, Senile Dementia, and Related Disorders: The Role of NIA" and "The National Institute of Mental Health Study" in Alzheimer's Disease, Senile Dementia, and Related Disorders
- "Drugs and Aging: Research, Education, and Cooperation" for Pharmaceutical Technology
- "Improving the Care of the Aged" for The Internist
- NIH Record articles:

Dr. Butler Appointed NIA Director  
Human Protection conference

World Health Organization meeting

Accidental Hypothermia

NIA Cultured Cell Repository

Professor Emeritus program

Ross McIntyre Award to Dr. Butler

NIA Board of Scientific Counselors

Physical Fitness conference

New Members of the National Advisory Council on Aging

Retirement of Dr. Solon

Dr. Shock Feted

Dr. Brody Appointed Associate Director, Epidemiology, Demography,  
and Biometry Program

Dr. Greulich Appointed Scientific Director

Dr. Pickett Appointed Associate Director, Extramural and  
Collaborative Research Program

Dr. Rapoport Appointed Chief, Laboratory of Neurosciences

Longevous Population of Vilcabamba workshop  
Mycoplasma Contamination Testing Service Contract

Miscellaneous projects undertaken by the Information Office ranged from the preparation of speeches, testimony, and press releases to the updating of the NIA sections of NIH publications. Specific projects are listed below:

- Testimony before the House Select Committee on Population
- Development of demographic charts illustrating the graying of America
- American College of Psychiatrists speech, questions, and handouts
- Remarks from Institute de la Vie (editing)
- Speech for N.C. Governor's conference (editing)
- Speech at Macy Conference on Care of the Aging (editing)
- Speech for Kingsbrook Jewish Medical Center
- Press Announcement: Gerontological Society Media and Aging Workshop
- Press Announcement: American Medical Writers Association
- Press Announcement: American Council of Life Insurance Executives
- Press Announcement: Physical Fitness and Aging conference
- Graphics for Medicine for the Layman speech
- Words on Geriatric Medicine for President's Commission on Mental Health (editing)
- Remarks at Epidemiology of Aging conference (editing)
- Remarks at Senile Dementia conference (editing)
- Remarks at Human Protection conference (editing)
- Press Announcement: Accidental Hypothermia
- Remarks at Black Aged symposium (editing)
- Chestnut Lodge speech (editing)
- Creation and Maintenance of a Clippings File
- Development of a Mailing List

- Press Announcement: American Association of Sex Educators, Counselors, and Therapists
- Press Announcement: Nutrition and Aging conference
- Press Announcement: Longevous Population of Vilcabamba conference
- Press Announcement: NIH Consensus Development Conference on Treatable Brain Diseases in the Elderly
- Backgrounder on senile brain disease
- Announcement of David Chicchirichi's Director's Award
- Speech for the American Psychiatric Association
- Speech for the Alvin Goldfarb Memorial Lecture
- Speech for the University of Minnesota conference on life extension
- Speech for Symposium at the 11th International Congress of Gerontology
- "Coming of Age: The Gray Revolution" -- general speech on geriatric medicine for use by all NIA staff
- Statement on "A Humanistic Approach to Our Last Days"

An NIA exhibit was also created, and was sent along with Information Office staff to the annual Public Health Service meeting, the Federation of American Societies for Experimental Biology convention, and the DHEW 25th anniversary celebration. The response to the exhibit was so positive that it was one of several that were asked to stay at DHEW for a few weeks following the celebration.

Two new Public Information Specialists were added to the Information Office staff, and a National Cancer Institute Expert Consultant is working in the Information Office on a part-time basis. Four of the Specialists received awards for their outstanding contributions to the NIA program of information dissemination.

The Information Office provided publicity for the NIA conferences described in the Director's report and prepared summaries of the nutrition, treatable brain diseases, and older woman conferences. The NIA Consensus Development Conference on Treatable Brain Diseases in the Elderly generated a high level of interest. Press coverage included a page one story in the Washington Post, a United Press International story that was picked up in at least 86 newspapers across the country, and articles in the New York News, Long Term Care, and Medical World News, among others. As a result of these articles and broadcast media coverage by WNBC Radio Network and WTTG-Metromedia TV, we have received at least 239 letters and telephone calls requesting



additional information in the two months following the conference. Calls from both the public and the press continue to come in, and it is anticipated that coverage of the conference and discussion of the issues it raised will remain in the news for some time.

A major press conference was held in December to alert the public, and particularly the elderly, to the danger of accidental hypothermia from prolonged exposure to even mildly cool temperatures. The brochure describing the condition, explaining who is at risk, and telling the elderly how to protect against accidental hypothermia was widely distributed during the winter in supermarket information racks. The story received wide coverage in both national and local media, and led to the addition of a warning to the elderly in the Department of Energy's "Tips for Energy Savers" booklet. Both major wire services--the Associated Press and United Press International--ran stories about accidental hypothermia, as did a variety of syndicated news services such as the Los Angeles Times News Service and the Newhouse News Service. In the two months following the conference, 214 news stories about accidental hypothermia were printed; since then, we have received about ten clippings a week on the subject, even during the summer months and from newspapers in southern states. Requests for additional information have come in from state health departments and energy offices, and also from a variety of organizations concerned with the elderly.

In addition, the brochure was the only one submitted by the Information Office to the 1977 competition of the Mid-Atlantic Chapter of the American Medical Writers Association. It won an award for "Meritorious Medical Writing."

In Fiscal Year 1978, the Information Office answered 3,050 written requests for individual publications and 1,027 requests for multiple copies of publications. Information Office staff answered 1,006 written inquiries and 3,640 telephone inquiries. Approximately 100,000 pamphlets, speeches, and booklets were distributed in response to individual requests, on mailing keys, and at meetings of organizations with an interest in aging and the aged.

Annual Report  
October 1, 1977 through September 30, 1978

Epidemiology, Demography, and  
Biometry Program (EDBP)

The functions of the EDBP commenced in October of 1977 when Jacob A. Brody, M.D., epidemiologist, joined NIA as Associate Director, EDBP. Our mission as the NIA focal point for quantitative population-based research on health and disease in the aging includes the disciplines of medicine, biostatistics, epidemiology, economics, sociology, and demography. The program also assumes the principal role for the Institute in multi-disciplinary epidemiological activities such as nutrition, prevention, clinical trials and environmental studies. Recruiting and planning occupied a considerable portion of the program's activity in FY 1978. The professional staff now consists of Clifford H. Patrick, Ph.D., economist-demographer; Joan Cornoni-Huntley, Ph.D., M.P.H., epidemiologist (expert appointment); and Yuko Palesch, M.S. (biostatistics). We have been fortunate in having Arthur White, Ph.D., Grants Associate; and Kirk Denicoff, first year medical student, Brown University, working with us this past summer. Our first year budget was \$195,000.

Our research will be conducted primarily by EDBP staff, supplemented and augmented by research contracts. Our three major areas of interest include: (1) Research data bases and methodologies; (2) Population-based research; and (3) Clinical epidemiology.

In the area of research data on aging, we plan to become a focal point for quantitative information, particularly from Federal sources, relating to morbidity and mortality, health services utilization, demography, economics, other social sciences, and environmental sciences. We are developing methods to utilize these data as they relate to research questions in the aging population. Population-based research will cover longitudinal studies as well as prevalence studies in selected populations. Clinical epidemiology will stress particular studies relating to health and include social and economic investigations.

Certain research concepts are emerging which potentially will cut across the three major program activities. One concept is the differential effect of economic status on health, particularly manifested by problems surrounding retirement and migration. Another is examining differences in terms of family structure, while the other multifaceted concept is to develop accurate information for survivorship of selected birth cohorts over age 65 by 5 year periods. This project will emphasize differences between sexes, ethnic groups, and regions in order to determine how these factors affect survival and to explore the functional use of these data in describing specific segments of the population.

During this fiscal year, several small professional services contracts were initiated in areas of research interest to EDBP, as described in the following pages, and one terminating project was transferred from ECRP to EDBP. The latter was "A Restrospective Study of Estrogen Usage by Postmenopausal Women

as it related to Hypertension and Coronary Risks," the Leisure World Study. Following a site review, it was determined to complete the current phase of the multi-year contract and not renew the contract beyond FY 1978.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Epidemiology, Demography, and Biometry Program

Contract Number: AG-6-2134

Contract Title: Aging, Estrogen Use, Hypertension and Myocardial Infarction

Contractor: University of California at Irvine, Irvine, California

Money Allocated: \$52,580 (FY 1978)

**Objectives:** The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of hypertension and myocardial infarction (MI) in a population of postmenopausal women living in a retirement community. The contract provides for support of a retrospective study of the incidence of hypertension and MI and in a specific and uniquely discrete population of 9,000 postmenopausal women. Cases of hypertension and MI occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified hypertension and MI cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting hypertension and MI.

**Significance to Aging Research:** Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risks of hypertension and MI in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for post menopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer, and stroke. There are not data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the millions of women over the age of 50 in the United States.

**Proposed Course:** The study was planned for a minimum of two years and will not be renewed in FY 1979.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Epidemiology, Demography, and Biometry Program

This is a professional services contract.

Contract Title: Nutritional Status of the Aged Adult Living in the United States.

Contractor: Karen Kinsell, M.S.

Money Allocated: \$840

Objectives: This contract involves preparation of the research on nutritional status of the aged adult living in the United States. This paper was presented at the NIA Conference on Nutritional Needs and the Health of the Aged Adult, June 5, 1978. The purpose of the paper was to analyze data from the first Health and Nutrition Examination Survey (Hanes 1) which collected nutritional and medical data on a national sample of the non-institutionalized population between the ages of 1 and 74. A major objective of the paper was to analyze the nutritional status of the aged population by race, sex, and income to determine if there were significant differences among these various socio-economic and demographic groups in their nutritional status. Among the measures of nutrition evaluated were: (1) caloric intake and body fat, (2) protein intake and serum proteins, (3) fat intake and serum cholesterol, (4) iron intake and blood iron indicators, and (5) vitamin A intake and serum vitamin A.

Significance to Aging Research: This research is significant because very little is known about the nutritional status of the elderly and the factors associated with nutrition such as income, race, or sex. The results of this paper should direct our future research into these areas and indicate the necessity of more extensive analysis of Hanes 1 and the later Hanes surveys. Since many of the diseases besetting the elderly may have a nutritional deficiency component, it is imperative that such research be carried out as a priority area.

Proposed Course: The study was completed and presented at the NIA conference on Nutritional Needs and the Health of the Aged Adult held on June 5, 1978.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Epidemiology, Demography, and Biometry Program

This is a professional services contract.

Contract Title: The Current State of Epidemiologic Research on Socio-Medical Risk Factors in Relation to Aging

Contractor: Judith B. Cohen, Ph.D.

Money Allocated: \$9,900

Objectives: Dr. Cohen will provide a systematic evaluation of the current state of epidemiologic research on socio-medical risk factors in relation to aging. The epidemiologic literature on aging has been reviewed to identify studies which meet standards for adequacy of research design and longitudinal research studies which may have either analyzed data or unanalyzed and unreported socio-cultural data as well as research studies which involve psycho-social risk factor determinations relating to specific disease outcomes such as cardiovascular diseases.

The evaluation will include conceptual and operational definitions of psycho-social, social and cultural risks which have been used, and the comparability for given factors in different disciplines as well as their reduceability. Psychological characteristics and social-cultural characteristics which have been found useful and adequate for epidemiological studies leading to meaningful associations with aging will be stressed. Longitudinal studies with adequate research designs for which the psychological and social-cultural characteristics could be applied will be documented.

Significance to aging Research: Social predictors of disease in the elderly is a small but diverse literature which has not been systematically evaluated. This evaluation will identify those psycho-social, sociological and socio-cultural risk factors which have been analyzed in previous studies and will provide directions for future research on social risk factor analysis. A substantial percentage of the elderly population is believed to be affected by psycho-social and socio-cultural impacts which may lead to illness or disease onset. The present research is significant in identifying the relationships which might exist to that extent. Once these relationships have been identified, steps can be taken to correct or minimize the risk and to provide social supports which should lessen the chance of disease outcome in the affected population.

Proposed Course: This study was completed within the fiscal year.



NIA Annual Report

October 1, 1977 through September 30, 1978

Epidemiology, Demography, and Biometry Program

This is a professional services contract.

Contract Title: Data and Methodology for Economic Research in Aging.

Contractor: Phoebus J. Dhrymes, Ph.D.

Money Allocated: \$8,000

Objectives: The purpose of this contract was to determine the appropriateness of the existing economic data for aging research and suggest ways of obtaining adequate information on the elderly. The contractor was also to assess the adequacy of existing statistical methods and quantitative models being used in aging research and indicate the most appropriate methodologies needed for human aging research and health research programs in general.

A report was prepared on the methodology of economic research in aging for the National Institute on Aging. The report included a discussion of both the micro-economic and macro-economic data and models appropriate for use in aging research. Specific attention was given to the dependent and independent variables, statistical methodologies, and quantitative models.

Significance to Aging Research: The role of economic factors and their relationship to the aging population are largely unexplored in a quantitative sense. This is partly due to the nature of the data which have been collected by federal agencies which are somewhat limited in their usefulness for examining economic hypotheses related to aging.

This contract pointed out the sources of economic data, the problems involved with the use of the existing data in examining hypotheses both at the micro-economic and macro-economic level and the areas in which current research can be most fruitful. This information is especially important to the National Institute on Aging as it prepares to undertake research on the relationship of economic factors and health and on the relationship of cost containment in health care services to the elderly.

Proposed Course: The study was completed within the fiscal year and a report from Dr. Dhrymes was delivered to the National Institute on Aging.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Title: Review of Literature Related to the Last Days of Life

Contractor: Adrian M. Ostfeld, M.D.

Address: 17 Marlboro Road, North Haven, Connecticut 06473

Money Allocation: \$1,000

Objective: The aim of this project is to review the medical, demographic, and social literature in relation to the last days of life and present an organized report. This information is required before specific studies can be undertaken on underlying and contributing causes to death.

Significance to Aging Research: There are many medical and social factors associated with the last days of life that are poorly understood. Knowledge of the circumstances surrounding death could be of assistance to families and societies as well as those persons providing health care in order to better cope with the occurrence of death. Medically related data needed includes: the presence of and amount of pain at time of death, types of medication or other treatment, prior illness, onset of the underlying cause of death, and whether death occurred during sleep or awake.

Demographic and economic information includes the place of death, if the death was in a hospital, how long the person was in the hospital, whether there was an autopsy, whether death was certified by a physician, and what was the cost of care and treatment. Social information includes: whether the death was expected and whether friends and family were present. There is a need for studies directed at better understanding the occurrence of death, but before such studies can be designed it is necessary to review the current status of knowledge in this area.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Title: Review of Information Concerning Human Leukocyte Antigen (HLA)

Contractor: Lowell E. Sever, Ph.D.

Address: 1538 Stanford, #7, Santa Monica, California 90404

Money Allocation: \$3,000

Objective: To review and evaluate information concerning theoretical and practical aspects of human leukocyte antigen and age associations. A report of this information is to be prepared to provide background information for planning future studies.

Significance to Aging Research: Before beginning a study of the relationship between HLA system and the process of aging, it is important to develop a clear understanding of the work that has been reported to date. It is necessary to review the literature on immunological aspects of aging and on the HLA system in aging. This would include a summarization of the data from those populations where HLA types have been presented by age. In addition, the literature on animal analogs should be reviewed.

In discussing the relationship between the HLA system and aging, attention should be given to population genetics and epidemiologic approaches. Genetic epidemiology will be considered as it relates to the study of possible differential selection of HLA types and the epidemiologic approach to this problem. A document that can be used for background data in the planning and development of future studies will be prepared.

NIA Annual Report

October 1, 1977 through September 30, 1978

Title: Conceptional Development of Population-based Research of Elderly People

Contractor: Dwayne Reed, M.D.

Address: 1601 Rose Street, Berkeley, California 94703

Money Allocation: \$1,000

Objective: The purpose of this contract is to review, by a site visit, past and current accomplishments of the aging projects being conducted by the staff of the University of California, Irvine and to consider potentials for future epidemiologic studies. This contract is also to include a report which presents the conceptual development of population-based research for studies of the epidemiology of aging.

Significance of aging research: There is a need for population-based epidemiologic research for studying problems of the aged. The project at the University of California at Irvine, supported by the National Institute on Aging, has used demographic and medical records of the Leisure World Retirement Community in Laguna Hills, California.

A site visit reviewed the past and current projects, the future studies planned, and gave recommendations for the future direction of the project as well as recommendations for future development of population studies of aging.

In addition, this contract covered a report to establish a conceptual framework for population-based research including the rationale for having a defined population, examples of the studies that could be completed within such a population, the type of communities and programs appropriate for study, and the qualifications and capabilities of applicants.

In summary, this contract is aimed at giving a complete overview of the concept of establishing a population for epidemiologic studies of the aged.





## NIA ANNUAL REPORT

### Report of Extramural and Collaborative Research Program

	<u>Page</u>
1. Overview of NIA's Extramural and Collaborative Research Program	ECRP-1
2. Basic Aging Program	ECRP-9
3. Molecular and Biochemical Aging Program	ECRP-21
4. Biophysiology and Pathobiology Aging Program	ECRP-31
5. Behavioral and Social Science Aging Program	ECRP-54



NIA ANNUAL REPORT  
OCTOBER 1, 1977 THROUGH SEPTEMBER 30, 1978  
REPORT OF ASSOCIATE DIRECTOR FOR EXTRAMURAL AND  
COLLABORATIVE RESEARCH PROGRAM

The Extramural and Collaborative Research Program (ECRP) provides support for research on aging through a variety of mechanisms: regular research grants, program project grants, research career development awards, research training grants, individual postdoctoral fellowship awards, special initiative awards, special research awards and research contracts. As NIA is the newest of the Institutes of the National Institutes of Health, its ECRP is still in the process of organizational change. Consequently, considerable effort was directed, during the reporting period, toward greater programmatic articulation and specification within ECRP; there was modest expansion of both funds and personnel and specific staff Program assignments for ECRP were publicly announced. Analytic capacity has been somewhat enhanced as the overall NIA's Program Analysis function has grown; program development efforts have continued; through: issuance of program announcements and requests for applications; appearances by staff at key professional society meetings; a variety of workshops, program consultations and conferences sponsored or co-sponsored by the NIA.

Each of the specific activities and initiatives alluded to above will be described in some detail in the sections immediately to follow. Succeeding sections of this Annual Report contain substantive accounts of activities within the specific ECRP Sub-Programs. These were prepared by the respective responsible staff officials.

Program Organization and Staffing

NIA's Extramural and Collaborative Research Program is in the process of replacing the former Adult Development and Aging Branch with the following substantive organization:

Basic Aging Program, with responsibility for development and support of research and research training in genetics and cellular aging genetics and comparative aging, theoretical gerontology, genetic and cellular resources and dermatology. Chief, Dr. Donald G. Murphy; Staff: Dr. Nirmal Das.

Molecular and Biochemical Aging Program, with responsibility for development and support of research and research training in immunology, pharmacology, intermediary metabolism and diabetes and aging. Chief, Dr. Lester Smith; Staff: Dr. Richard Irwin.

Biophysiology and Pathobiology Aging Program, with responsibility for development and support of research and research training in nutrition and metabolism, endocrinology and hormone dynamics, neuroscience, vertebrate models and resources. Areas such as research geriatric medicine, exercise, physiology, epidemiology and prosthetics are subsumed under these categories. Chief, Dr. Don C. Gibson, Staff: Dr. Michael Dieter and Dr. Zaven Khachaturian.



Behavioral and Social Science Aging Program, with responsibility for the development and support of research and research training in problem areas such as family structure and life-style patterns and aging, sociocultural and ethnic factors in aging, work, retirement and leisure, cognition, learning and memory, behavioral processes in adaptation to aging. Acting Chief, Dr. Betty H. Pickett, Associate Director, ECRP; Staff: Mrs. Shirley Bagley and Dr. Walter Spieth.

The foregoing is regarded as an interim arrangement, subject to refinement and revision prior to final formulation of what is anticipated to become a Branch structure.

Four professional staff and coordinated support personnel have been added during Fiscal Year 1978, bringing the ECRP complement to a total of 24 persons.

#### Program Statistics

Table I shows that awards in the amount of \$26,533,000 are projected for the total period, October 1, 1977 through September 1978. Most, by both number and dollar amount, are for the traditional, investigator-initiated awards. Sixty-two percent of the NIA's funds are projected for expenditure primarily on these and other types of individual investigator-oriented awards. On the other hand, only 36% of the funds are directed toward program projects and core center grants (NIA currently supports only a single core center grant). Research contracts, interagency agreements and research training awards continue to account for only a modest part of the overall ECRP expenditure. (17%)

Table II shows the distribution of funds expended through NIA's Extramural and Collaborative Research Program by type of award (new award, competing renewal award, supplemental award) versus non-competing or previously committed renewal awards. The data clearly indicate that NIA's funds continued to be heavily obligated for the continuation of awards reviewed and initiated in prior fiscal years. Close to two thirds of the extramural grant funds are devoted to continuations, reflecting the effects of the increased number of new awards started in Fiscal Year 1977. On the other hand, the dollar amount available for new awards in fiscal '78 was only 24% of the total ECRP expenditure, versus the 1977 situation where 41% of the total dollars were for new awards.

#### Substantive Analysis of Extramural and Collaborative Research Program Awards

Although the Extramural and Collaborative Research Program is in the process of developing a sophisticated programmatic structure, program analysis capability is still not sufficiently refined to present statistics based on the new breakdown described above. However, within each of the sub-program reports to follow, we will provide an indication of the extent and nature of support for each. A rough categorization by disciplinary area is shown in Table III, where broad areas are described. As can be seen, the ECRP Program content continues to be heavily biological (61%). Only a relatively modest proportion (9%) of the ECRP funds are expended for clinical research and research training activities; behavioral and social science research and research training in aging account for some 25% of the overall Fiscal Year 1978 expenditure, about the same proportion of the total as in Fiscal Year 1977.

## Applications Reviewed by the National Advisory Council on Aging in Fiscal Year 1978

Table IV shows that a total of 673 applications was reviewed by the National Advisory Council on Aging (NACA) in Fiscal Year 1978; of these proposals, 529 had primary assignment to NIA (that is, the Division of Research Grants, which assigns applications to NIH Institutes on the basis of their relevance to the respective Institutes' missions judged 529 to be focally related to aging). The remaining 144, judged by the Division of Research Grants to be of secondary relevance to NIA's mission (and of primary relevance to other Institutes), were given only secondary assignments to NIA. Approval rates for primarily assigned applications were within the average range for NIH Institutes. The total number of applications reviewed in Fiscal Year 1978 (673) contrasts with the 475 reviewed by NACA in Fiscal Year 1977, showing close to 200 additional Council actions in 1978. The major portion of the increase was in applications primarily assigned to NIA, (529 in FY 1978 versus 363 in FY 1977). The 144 proposals secondarily assigned in Fiscal Year 1978 contrast with the 112 such proposals of Fiscal Year 1977.

Study Section Review for NIA's applications was spread, as in FY 1977, across more than 40 of the Division of Research Grant's 52 Study Sections. NIA maintains a continuing concern, of course, for the quality of its research grant applications in all the many fields of biomedical and behavioral/social science involved.

The NIA's Division of Research Grants (DRG) has encouraged efforts to broaden Study Section sensitivity to the complex of issues surrounding research in gerontology. To this end, NIA's ECRP professional staff met with DRG's Executive Secretaries to discuss issues surrounding the NIA's new Special Initiative Awards, launched this year (described below under program development efforts). In addition, DRG took the initiative in holding a special one-day Symposium on Aging, sponsored by two Study Sections (Molecular Cytology and Pathobiological Chemistry) was held in March 1978. The purpose was to highlight for the Peer Review group members salient substantive and methodological issues in the biology of aging.

The increase in professional staff in ECRP, which occurred in FY 1978, will enhance NIA's ability to maintain closer ties with Study Sections and to continue and enhance the promising liaison initiative indicated above.

## Program Development Efforts

Refinement of NIA's referral guidelines has continued since their drafting in 1977. With appropriate ECRP Chiefs, the Associate Director has pursued agreements with several of the NIH Institutes concerning assignments of applications within various areas of respective overlapping interests. To date, Memoranda of Understanding have been negotiated in specific areas with NIAID, NIGMS, NINCDS and NICHD. Additional understandings focussed on the complex of overlapping issues in the Behavioral and Social Sciences as related to Aging, are presently in process of negotiation with NIMH (an ADAMHA Institute).

## Issuance of New Announcements and Revision of Prior Announcements

NIA has prepared for reissue in the NIH Guide, revisions of the Institute's prior program announcements in Pharmacology and Aging, and Nutrition and Aging,

(issued originally, respectively, in March and August 1977). New versions of those announcements will be published in the NIH Guide prior to the end of Fiscal Year 1978, along with new program announcements concerning both the Basic Aging and Behavioral and Social Sciences Programs.

NIA's Special Initiative Award, a new type of grant, designed to provide opportunity for exploratory and pilot activity for investigators and institutions wishing to undertake gerontological research, has been announced twice during Fiscal Year 1978, most recently in the NIH Guide for May 12, 1978. Although only a modest response from the science community has been received by the Institute so far, six such awards were made in Fiscal Year 1978. In addition, FY 1978 NIH Guide announcements are scheduled for two new types of awards (Geriatric Medicine Academic Award; NIA Clinical Investigator Award) designed to encourage research in various areas of geriatric medicine. Furthermore, NIA was a participant in issuance of a joint announcement issued in Fiscal Year 1978 by eight NIH Institutes soliciting research grant applications in the general area of Diabetes Mellitus and related problems.

#### Program Development Meetings

As in Fiscal Year 1977, NIA continues to hold a variety of program development meetings with the aims of obtaining scientific advice on program direction, assessments of state-of-the-art, and stimulation of additional research on aging. As reported earlier, some of the activities involve partial support for scientific meetings or symposia organized outside the Institute, others represent informal consultations, working conferences or larger scale, more formal public meetings. Some of the meetings result in published reports of proceedings, others in less formal reports. In many of these instances, the strategy has been to bring together with established investigators in aging, persons who have not yet undertaken gerontological research, but whose substantive interests to date, appear to have promise for furthering goals of gerontological research. The hope is, of course, to stimulate interest by a wide scientific community in the NIA's attempt to broaden the science base for the complex of fields which contribute to gerontological research. The several program development meetings held during Fiscal Year 1978 will be outlined within the substantive reports of activities of each of the four sub-program areas contained in the succeeding sections of this Annual Report.



TABLE I  
DISTRIBUTION OF ECRP FUNDS  
October 1, 1977 - September 30, 1978  
(estimated)

	NUMBER OF AWARDS	(in thousands) AMOUNT*	PERCENT
RESEARCH			
Program Projects & Core Center Grants	26	7,875	36%
Research Projects (traditional)	173	12,247	55%
Scientific Evaluation	1	108	1%
Conferences	1	21	1%
Research Demonstration & Dissemination Projects	1	121	1%
Exploratory Grants	6	447	2%
Special Research Awards	26	938	3%
Modified Research Career Development Awards	7	228	1%
Sub-Total	241	21,985	100%
TRAINING			
Graduate Training Programs	4	497	21%
Institutional National Research Service Awards	18	1,408	59%
Postdoctoral Individual National Research Awards	33	485	20%
Sub-Total	55	2,390	100%
RESEARCH CONTRACTS	17	1,930	89%
INTERAGENCY AGREEMENTS	2	228	11%
Sub-Total	19	2,158	100%
GRAND TOTALS	315	26,533	

\*Funds for supplemental awards and supply allowances are included, but are not counted in number of awards



TABLE II  
DISTRIBUTION OF ECRP FUNDS  
October 1, 1977 - September 30, 1978  
(estimated)

TYPE OF AWARD*	NUMBER OF AWARDS	(in thousands) AMOUNT	PERCENT
New	99	6,318	24%
Competing Renewals	24	2,824	11%
Noncompeting Renewals	194	17,130	64%
Supplements	<u>14</u>	<u>261</u>	<u>1%</u>
TOTALS	315	26,533	100%

\*Includes contracts and interagency agreements.

TABLE III

SUMMARY BY MAJOR CONTENT AREA  
GRANTS/CONTRACTS  
(estimated)

October 1, 1977 - September 30, 1978

	(in thousands)	
	AMOUNT*	PERCENT
RESEARCH		
Biological	13,507	61%
Clinical	1,884	9%
Behavioral	2,923	13%
Social	1,483	7%
Multicategorical	<u>2,188</u>	<u>10%</u>
Sub-Total	21,985	100%
TRAINING		
Biological	869	37%
Clinical	146	6%
Behavioral	501	21%
Social	166	7%
Multicategorical	<u>708</u>	<u>29%</u>
Sub-Total	2,390	100%
CONTRACTS		
Biological	1,458	75%
Clinical	163	9%
Behavioral	- -	- -
Social	187	10%
Multicategorical	<u>122</u>	<u>6%</u>
Sub-Total	1,930	100%
INTERAGENCY AGREEMENTS		
Biological	8	3%
Clinical	- -	- -
Behavioral	- -	- -
Social	- -	- -
Multicategorical	<u>220</u>	<u>97%</u>
Sub-Total	228	100%
GRAND TOTAL	26,533	

\*Funds for supplemental awards and supply allowances are included, but are not counted in number of awards.

TABLE IV

Applications Reviewed by the National  
Advisory Council on Aging  
October 1, 1977 - September 30, 1978

		Applications Reviewed	Approval Rate (%)
October 1977			
	Primary	167	57%
	Secondary	<u>52</u>	48%
	Total	219	
January 1978			
	Primary	148	65%
	Secondary	<u>46</u>	54%
	Total	194	
May 1978			
	Primary	214	52%
	Secondary	<u>46</u>	61%
	Total	260	
	GRAND TOTAL	673	

Source: Summaries of Council Recommendations for October 1977,  
January 1978, and May 1978 provided by the Grants  
Management Office, NIA.

## BASIC AGING PROGRAM (BAP)

Within this program area the NIA plans, implements and supports research in the areas of Genetics and Cellular Aging, Genetics and Comparative Aging, Theoretical Gerontology, Dermatology, and Genetic and Cellular Resources. This support is for fundamental molecular and genetic research on the mechanisms of aging at the cellular level; on invertebrate organisms and plants; through theory elaborated by abstract modeling; and on skin. In support of these four research areas, the BAP funds development of characterized biologic resources, and funds research training and services related to the use of these biologics.

Genetics and Cellular Aging: This area currently supports 45 grants. The capacity to study human cells independent of the individual has been emerging over the past several decades via the relatively new technologies of cell, tissue, and organ culture. Cell culture technology now enables us to perform experiments which would not be possible to perform in man. Although it is not altogether clear how in vitro cells relate and correspond to in vivo cells, these technologies do, in fact, provide remarkable access to basic knowledge of cell structure and function. Cell culture technology is quickly becoming a prominent tool for gerontologists studying cellular aging.

Emergent from cell culture technology is the field of somatic cell genetics which has contributed to our knowledge and understanding of chromosomal sites, structure and function of specific human genes. For the gerontologist, somatic cell genetics is a tool to investigate the genetic control over cellular senescence.

We hope to gain knowledge of cellular senescence and the genetic manifestations of cellular aging from studies of populations of cells implanted into host animals. In such studies the host laboratory animals (usually mice or rats) provide the "culture" environment. Distinct from such transplant studies, but of similar potential are chimeric and genetic mosaic animals. Chimeric animals enable observation of development and senescence in cells of different genetic heritage within the normal tissue arrangement of one animal. This is made possible through recently developed technologies to implant genetically distinct cells into host organisms during the very early embryological stages. Genetic mosaics are animals which have different phenotypic expressions of the same cell types due to varying gene dosages. Mosaic animals will be sought which express different rates of cellular aging in the same cell type.

The Genetics and Cellular Aging component of BAP encompasses grant supported studies on the mechanisms of cellular aging utilizing the technologies of cell culture, somatic cell genetics, cell



and tissue transplantation, chimeric and genetic mosaic biology. Current research, by such grantees, ranges from investigations to determine control factors involved in cell proliferation of cultured human diploid cells to the biochemistry of cell membranes.

Findings on NIA supported investigations in the area of Genetics and Cellular Aging in FY 1978 include:

Membranes:

1. A reduction in steroid hormone receptor sites in WI-38 and IMR-90 cells. Such hormone, for example hydrocortisone, is known to increase the proliferative ability of cells.
2. Age-dependent decrease in post-synaptic biogenic receptors. In striatum and cortex of rabbit brain dopamine-stimulated adenylate cyclase activity decreases as the animal becomes older.
3. Increase in the membrane-bound -glutamyltransferase activity with the increase in in vitro cellular age. This enzyme is involved in the transport of amino acids across the membrane.
4. Enzymatic removal of some surface glycoprotein components of cultured human cells leads to an increase in norepinephrine-stimulated adenylate cyclase activity. This also leads to an increase in the cell cycle time.
5. Alteration in the distribution of intramembrane particles.
6. Number and size of gap junctions decrease in aging cells.
7. Changes in membrane fluidity as revealed by clustering of concanavalin A binding sites.
8. Metabolic parameters at or near the surface, responding to messages from outside, are altered in aging cells. These metabolic alterations seem to include the cAMP system (increased basal cAMP) and their response to prostaglandins.

Somatic Cell Genetics:

1. Several Chinese hamster mutant cell lines, defective in purine and/or pyrimidine metabolism, have been obtained. These mutants and heterokaryons, obtained using these mutants, will be used to study regulation of purine and pyrimidine metabolism in cells.
2. Test crosses with cells (somatic cell hybrid) of long and short life span show intermediate life span. This indicates that

mutational theory of in vitro aging may not be correct. Most mammalian cells stop dividing in vitro because they undergo terminal differentiation.

#### Cell Life Span:

1. Hydrocortisone increases the in vitro life span of WI-38 cells, perhaps allowing cells to utilize serum factors more efficiently.
2. Smooth muscle cells (2 types) obtained from uterine scrapings are diploid and have finite (40 PDL) life span.
3. Keratinocytes can be cultivated, with the use of 3T3 feeder layer cells. These cells differentiate in culture, and they have finite life span.
4. Skin fibroblasts have shorter in vitro life span than lung fibroblasts.

#### Chromosomal Abnormalities:

1. The short-lived renal epithelial cells and long-lived lung fibroblasts exhibit similar degree of chromosomal abnormalities. Increase in polyploidy and chromosomal abnormalities seen as cell approached phase out.
2. Using BuDr-differential sister-chromatid staining procedure, it was observed that kidney epithelial cells divide more rapidly than lung fibroblasts.

Genetics and Comparative Aging: This program currently supports 20 grants. We do not yet understand the basic mechanisms responsible for longevity and senescence in man, other mammals or even simple forms of life. The known strategies of biological structure and function at the cellular level are remarkably similar in all forms of life. This observation suggests that the processes responsible for senescence at the cellular level have important implications for all organisms. However, the great diversity of organisms and the extreme range of conditions under which populations of animals and plants maintain themselves and evolve, offers the likelihood of differing bases for expression of longevity and senescence at the organism level. Fundamental knowledge of the events leading to senescence in any organism will provide information to guide future theory and experiments to increase our understanding of human aging. Within the BAP component on Genetics and Comparative Aging research is supported on laboratory invertebrate animals, plants, and prokaryotes that express senescence. These laboratory organisms are selected for desirable features to the experimentalist. These features often include favorable characteristics for genetic studies. For the gerontologist, knowledge of genetic control over maintenance of fitness and of longevity is of major importance.

Mutant lines of experimental organisms which express differential senescence and longevity could enable localization of the fundamental mechanisms responsible. Genetic dissection of organisms to explain senescence is difficult to achieve as the causes of senescence are likely to involve interactions of multiple genes. This remains, none-the-less, a most promising avenue for gerontology. Currently, there are NIA grantees pursuing such studies with the fruit-fly, Drosophila, the laboratory nematode, Caenorhabditis elegans, the slimemold, Dictyostelium, the "colonial flagellate", Volvox, and the protozoan, Tetrahymena. Basic aging studies on these and other organisms are also pursued independent of genetic experiments. The research encompasses molecular genetics and the biology of aging at the subcellular, organismic and population levels.

Findings on NIA supported investigations in Genetics and Comparative Aging reported in FY 1978 includes:

1. In aging housefly mineral rich lipofuscin accumulates in cells; the rate of accumulation is higher in short-lived flies. This pigment accumulation is related to the physiological state of flies, rather than their chronological age.
2. In the flight muscle of aging house flies, mitochondria seem to fuse. There is a decrease (15-20%) in contractile components and increase in autophagic vacuoles.
3. Recent studies indicate that many age-associated changes in mitochondrial functions described earlier, probably are not valid because of the use of unsatisfactory techniques of mitochondrial isolation. Some mitochondrial functions, such as phosphate carrier function of inner mitochondrial membrane (of insect flight muscle cells) may be affected during aging.
4. Marked changes occur in tissue levels of PG-E (prostaglandin-E), PG-F, and PG-B, cAMP and cGMP during growth, development, and senescence in nematodes.
5. Two types of myosins are seen in nematode muscles. In mutants in which muscles are aberrant, myosins show many normal properties, it seems possible that some genes are involved in the formation of precise architecture of myosin in muscle cells. These genes are affected in mutants.
6. Conformation of change in enolase in older nematodes.
7. Synchronization of nematodes (of the same age) with the use of FuDr.
8. Studies show age-related changes in nematodes: cessation of gonad function, gonad atrophy, pigment accumulation in intestine, increased transparency of tissues, increase in sluggishness, partial paralysis and death.

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9. At 20 C the nematode dies in 15 days, if grown in media containing bacteria. If the temperature is increased to 25 C, death occurs by 12 days. If grown in chemically defined medium, worms can live 21-25 days at 20 C.
10. The pentose of RNA serves as a source of glucose equivalents for end product saccharide (cellulose, trehalose) synthesis in Dictyostelium discoideum.

Theoretical Gerontology: This area currently supports 2 grants. All NIA grant programs incorporate theory development in research observations; experimental design and data interpretation. This theoretical aspect of BAP emphasizes computerized and mathematical models of life systems and processes of significance to gerontology. Such systems and processes range from molecular processes and biochemical events to population genetics.

NIA opportunity to support grants in theoretical gerontology has been very limited, partly due to a lack of interest by the scientific community (as expressed through grant applications). The Institute recognizes that interest in this field probably will increase and anticipates the contribution that such theoretical work holds for experimental gerontology.

Current grants in this program support computerized modeling of biochemical events associated with aging and development in the slime-mold, Dictyostelium.

Findings on NIA supported investigations in Theoretical Gerontology reported in FY 1978 include the following:

The kinetic model of carbohydrate metabolism in Dictyostelium discoideum now consists of 20 reactions and 125 parameters. One of the predictions of this model is that glucose is compartmentalized in this organism. Experimental verification of this prediction is now available; a very high concentration of glucose is seen in the major cell type (spores) during culmination.

Dermatology: This area currently support 3 grants in Dermatology. People take pride, and find comfort in youthful looking and functioning skin. To this end, individuals spend vast sums of money each year. The conditions often associated with aging skin, (dryness, chronic itching, the undesired appearance of "aging") interfere markedly with the quality of life among many mid-life and older persons. This detracting from quality of life is obvious not only by cosmetic detriments, but also by the social stigma, "ageism", consequent to communicating age through appearance of the skin.

The public's concern with the problems of aging skin recently lead to renewed interest in geriatric dermatology research. Very little



is known about the basic biology of aging skin, much less the underlying mechanisms for its senescence.

The study of skin aging is closely related to some BAP supported research on cellular senescence and, in particular, to in vitro cellular senescence. Skin is the most accessible organ. Skin biopsies are routinely and easily performed for study of cells in culture. New techniques are available to study the turnover of certain prominent skin cells (keratinocytes) including collection of cell remnants which slough off normally at the surface of the skin. The extracellular components of skin are easy to analyze as are many measurements of skin function.

Because the Genetics and Cellular Aging component of BAP encourages use of skin-derived cell cultures, there is recognized overlap with the Dermatology effort.

Findings on NIA supported investigations in Dermatology reported in FY 1978 include the following:

Human foreskin keratinocytes can now be cultured in a medium in which 3T3 cells have been grown previously. In such a conditioned medium, keratinocytes underwent 30 population doublings and synthesized keratin. It has also been possible to culture keratinocytes in unconditioned medium, supplemented with hydrocortisone and bovine pituitary extract.

Genetic and Cellular Resources: This area currently supports 3 grants and 2 contracts. Fundamental to gerontology research is the availability of high quality, well-characterized biologics. Such resources are of considerable value to laboratories with limited funds and lacking expertise in quality control procedures. This portion of the BAP is responsible for biologics supply, characterization, and support services for cell culture and invertebrate, plant, and prokaryote systems. The cell culture services support not only the Genetics and Cellular Aging component of BAP, but also other Programs of the ECRP, and the NIA Intramural Research Program at the Gerontology Research Center in Baltimore.

The Genetic and Cellular Resources component is the focal point of BAP contract activity, although all grant mechanisms are accessible as necessary.

Current support includes research on cell-lineage and population characteristics of human diploid cells in culture; the establishment, isolation and characterization of longevity-mutant nematodes and training of postdoctoral scientists in the technologies of tissue specific cell-line development.

There are two contracts supported under this area of BAP: 1) the NIA Cell-Line Repository supports acquisition, characterization

and distribution of cell-lines of special utility to NIA grantees, prospective grantees and other gerontologists, and 2) for the same group of investigators, the Mycoplasma Infection Testing Service is a contracted resource contributing to quality control in cell culture laboratories.

Findings on NIA supported investigations in Genetic and Cellular Resources reported in FY 1978 include:

1. Growth of human foreskin fibroblasts in culture has been recorded by the time-lapse photography. The photographic images were then processed by computers and following information obtained:
  - a) all cells finally stop dividing; cells which leave the cycle do not die;
  - b) the loss of all division is not due to contact inhibition.
  - c) some daughter cells from a founder cell stop dividing before others.
  - d) there is a lack of cell division synchrony between daughter cells and this asynchrony increases with the age of the culture.
  - e) cells tend to follow tracks of other cells; large non-dividing cells move as fast as small dividing cells, but not as often.

Based on some of these results, it has been suggested that the loss of the proliferative ability of cells is an indication of "normal" cell differentiation rather than cellular senescence.

2. The NIA Cell-Line Repository at IMR has banked about 180 different human cell lines, which include lines from: skin tissues of both sexes and of different ages, lung tissues (IMR-90 and IMR-91), individuals with various genetic diseases and growth disorders, normal cell lines transformed by virus infection, tumor cell-lines and cell-lines from skin of patients receiving radiation and chemotherapy. In addition, the NIA Cell-Line Repository have provided cytogenetics services on six different cell lines to NIA grantees during the past year.
3. The Mycoplasma Testing Service contract, awarded to IMR on April 1, 1978, has been testing, for Mycoplasma infection, cultures supplied by NIA grantess and investigators from GRC.

## Basic Aging Program Workshops

**TITLE:** The Use of Differentiated Cells in Culture for Aging Research  
(May 1978)

**Staff Organizer:** Donald G. Murphy, Ph.D. in collaboration with  
Warren W. Nichols, M.D., Ph.D.

**Support:** as an integral part of the contracted resource "NIA  
Cell-Line Repository," Institute for Medical Research,  
Camden, N.J.

**Summary:** Formed presentations and discussions covered cell culture  
systems of differentiated cells of potential value to  
gerontology. These culture systems included: keratinocytes,  
winay draeb epithelium, thyroid, teratocarcinoma,  
chordocytes and neurons.

## Basic Aging Program Publications

1. 1977, Nichols, W.W. and D.G. Murphy ( EDS.). Senescence: Dominant or Recessive in Somatic Cell Crosses. Plenum Press, N.Y. 123 pages.
2. 1977, Nichols, W.W. and D.G. Murphy (EDS.). DNA Repair Processes and Cellular Senescence. Symposium Specialists, Miami, Florida 286 pages.
3. 1978, McGarrity, G., D.G. Murphy and W.W. Nichols (EDS.). Mycoplasma Infections of Cell Culture. Plenum Press, N.Y. 342 pages.
4. 1978, Murphy, D.G., N.K. Das, L.A. Pohutsky, and I.J. Fred. The Basic Aging Program of the Extramural Collaborative Research Program, National Institute on Aging. Experimental Aging Research:
5. 1978, Das, N.K., D.G. Murphy, National Institute on Aging Cell-Line Repository. Experimental Aging Research:
6. 1978, Das, N.K., D.G. Murphy and I.J. Fred, National Institute on Aging, Mycoplasma Testing Service. Experimental Aging research:

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative Research Program

Contract Number: N01-AG-4-2865

Contract Title: Selection, Production, Characterization and Distribution  
of Genetically Marked Cells for Aging Research

Contractor: Institute of Medical Research, Camden, New Jersey  
(Principal Investigator): Dr. Warren W. Nichols

Money Allocated: \$178,657

Objectives:

1. Provide standard, highly-characterized, extensively banked lines of normal human diploid fibroblast-like cells.
2. Provide banked and characterized, genetically marked or mutant cells identified as possessing features of value in probing mechanisms of cellular aging.
3. Provide standard, characterized, moderately banked lines of tissue-specific cell types.
4. Contribute to the development of the field of cellular aging theory, concepts, and techniques, through consultation and workshops, particularly in the areas of somatic cell genetics and cytogenetics.
5. Limited cytogenetic screening for aging research.

Significance to Aging Research:

Events at the cellular level are probably major determinants of the expression of longevity and senescence at the level of the organism. The techniques of cell-culture enable investigation of cellular events consequent to aging independent of the complexity of the whole organism. Such in vitro studies are conducted on cells as they age in the cell culture environment, and also, on cells derived from people and experimental animals of different ages. Most such studies to-date are on fibroblast-like cells. Advances in cell-culture technologies show considerable promise that an increasing number of tissue-specific cell types, that is, differentiated cells in culture, will become available to the gerontologist for study. The contractor is to provide leadership in the acquisition, banking and distribution of differentiated cell lines valuable to the gerontologist. The contracted resource also supports NIA interest



in knowledge of genetic mechanism of cellular aging approached through the use of genetically marked cell-lines and use of the techniques of somatic-cell genetics.

An extensive number of cell lines have been accepted and distributed by the repository. The standard cell line (IMR-90), in particular, has found extensive usage not only in gerontology, but by the biomedical research community, in general.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative Research Program

Contract Number: N01-AG-8-2117

Contract Title: National Institute on Aging Mycoplasma Contamination Testing Service

Contractor: Institute for Medical Research, Camden, New Jersey  
Principal Investigator): Dr. Gerard J. McGarrity

Money Allocated: \$47,572

Objectives:

1. The detection, prevention, and control of Mycoplasma infection as a service primarily to NIA grantees and NIA Intramural Laboratory
2. Provide consultation to laboratories having chronic problems with mycoplasmas and other micro-organisms.
3. Distribute a quarterly newsletter on quality control of cell cultures.

Significance to Aging Research:

The NIA encourages the use cell-culture technologies to pursue knowledge of genetic, molecular and cellular mechanisms of human aging. Investigators working with cultured cells must exercise extreme caution to avoid microbial contamination of those cultures. Gross microbial infections due to bacteria or fungus usually are obvious and rapidly destroy the contaminated culture. It is the more subtle infections, as with Mycoplasma, which may go undetected. Mycoplasmas, the smallest known free-living organisms, are common contaminants of cultured cells. They are difficult to detect, and, while their presences may not destroy the host cell-culture, it often alters the metabolism and function of the cultured cells. Unlike bacteria, mycoplasmas lack cell walls and are resistant to many common antibiotics. Mycoplasma contamination is of particular concern in gerontological research, in which it is often necessary to maintain cell-cultures for a relatively long period of time. During the initial reporting period for this testing. The calculated Mycoplasma-infection rate is slightly over 10%.

## MOLECULAR AND BIOCHEMICAL AGING PROGRAM

### Immunology

Since immunologically incompetent individuals are very susceptible to infections, immune dysfunction is probably one of the major sources of health problems of the elderly. The pathogenesis of a variety of age-related diseases may have an immunological basis. The overall aim of the research program in Immunology is to define the immunologic impairment in aging animal models and man.

Since its inception in 1974 research on immunology and aging has reached a modest level of activity. Presently there are 37 research and training grants, fellowships, and contracts. The budget is approximately \$2.6 million.

Current studies are related to the basic mechanisms of immune cellular senescence, to the influence of genetics on immune expression, on the mechanisms of immunoregulation and immunodeficiency, and on the immunological basis of various pathologies.

Research is underway on a variety of topics including: the influence of controlled antigenic stimulation on the immune potential with special emphasis of aging on germ free conventional mice; possible relation of aging to the effects of long term histocompatibility reactions arising in the body; the nature of the impaired humoral immunity in mice; the contribution of thymic involution to impaired immunity; the capacity of thymopoietin and other thymic factors to preserve or reconstitute senescence of the immune response; the effect of caloric under nutrition on life span, immune function and age-related diseases; suppressor T cell activity; the apparent loss of B lymphocyte function during aging; a determination of the extent of macrophages in the age-related decline in T cell response; the role of enzymes involved in lymphocyte biochemistry; the changes in lymphoid tissue cells which allow and/or induce the development of anti-Ig responsive B cells; the pathogenesis of amyloid; autoimmunity.

Other projects include the selection of variant-mutant cells for Ig production in human lymphoblastoid cell lines in old and young patients with progeria and the characterization of a variant Ig gene product. Still other studies underway attempt to determine if the ease of tolerance induction may provide insights into the mechanism of the autoimmune phenomena which increases in incidence with age. Various pharmacologic reagents such as prostaglandin are being used to study immunoregulatory activities in young and old humans.

The modest activity within the Immunology Program has allowed for the evolution of research problems related to several fundamental possible etiologies of immune dysfunction during the aging process. Some studies will enhance our understanding of the influence of the environment on development and maintenance of immune function throughout life. These studies may indicate ways to optimize the performance of the immune system achieving life long protection of the host. Other studies may have a direct bearing on health problems associated with autoimmune diseases and neoplasms. Other studies are being directed towards better understanding and testing of the effect of caloric

under nutrition. The studies have resulted in quite a significant prolongation of the life span of long lived strains of mice. It seems that suppressor T cell activity increases with age and is therefore an important age-related immunologic lesion. There is some preliminary evidence of the appearance of hyperactive and auto-reactive B cells in aged humans. The Molecular and Biochemical Aging Program seeks to expand the total research effort related to immune dysfunction during aging. Workshops, program announcements, active staff programming, and other vehicles and mechanisms will be employed to recruit competent immunologists into the field to study the many outstanding and significant problems being investigated or worthy of immediate attention. Planning for the major workshop on immunology and age to be held in the next fiscal year was undertaken during Fiscal Year 1978.

#### Pharmacology and Aging

In 1974 the per capita health cost for an older person was nearly three times that for a younger adult. A substantial proportion of that cost is due to expenditures for medication. It has been estimated that the elderly who comprise approximately 11% of the population use about 25% of the drugs produced. Relatively little is known about changes in drug effects in relation to the age of the individual and yet drugs are being administered more extensively to patients as they grow older. It is obvious that with increasing age there is a greater likelihood of illness, and therefore, a greater need to use therapeutic agents frequently on a chronic basis.

Drugs may behave differently in the aged than in the healthy young individual on whom most information has been acquired. Because of the many physiological, and perhaps pathological, alterations occurring with age, it is essential that basic and clinical research be undertaken to evaluate the safety and efficacy of drugs being administered to the elderly. The possibility of undesirable pharmaceutical complications such as adverse drug reactions and drug-drug interactions may occur more frequently. Physiological impairment of the heart, blood vessels, kidneys, digestive tract, and nervous system among other organs are prevalent in the elderly individual. It is hoped that an NIA-supported program in pharmacology and aging will illuminate the aging processes as well as provide leads to more effective therapies. Only a modest program on pharmacological problems related to drug behavior in the aging system is underway now. The following aspects are being studied: measurements of ventilatory control attributable to carbon dioxide before and after anesthesia and surgery in elderly subjects; studies on the several behavioral effects of dopamine agonists drugs before and after chronic administration of neuroleptic (anti-psychotic) drugs in young, mature, and old-age rats; studies on the effects of biogenic amines on MAO activities; studies on the effects of age on an organism's sensitivity and responsiveness to drugs, especially those used in therapy of the cardiovascular and central nervous system; studies on the quality and quantity of receptors in nerve cells as the animal ages; studies on the sensitivity of the aging organism to a wide variety of new and old drugs used to correct abnormalities in heart rhythm (anti-arrhythmics); a systematic re-examination of data to evaluate whether or not certain drug interactions tend to be more common in the elderly. Because the existing program was begun only recently and on a limited basis, significant results are not yet available.

Areas judged to be in need of further development include: drug interactions involving those organs known to undergo the most dramatic and important changes



during aging, i.e., the brain, heart, kidney, gastrointestinal tract, and liver; changes in response to anesthetics and pain killing drugs; research on the sociological and cultural aspects of drug use and abuse in the elderly; longitudinal studies, particularly of environmental factors; drug absorption, rate of metabolism, and rate of elimination using prototype drugs with various organs known to undergo pathological changes during aging; drugs used in the prevention and treatment of cardiac problems such as digitalis, anti-arrhythmics, and anti-hypertensives; influence of age on the efficacy and toxicity of hypnotics and barbiturates; nutritional factors which affect drug efficacy and toxicity; efficacy and toxicity of hormones and hormone antagonists, and of antibiotics; neuropharmacological effects of drug use and abuse among the elderly; epidemiology of drug use and abuse and physician practices involving the elderly; studies on the development of unique animal models of pharmacological research.

### Intermediary Metabolism

A proper understanding of the aging process requires knowledge about age-associated changes occurring at the biochemical level. Indeed, many of the more obvious changes in organ systems and physiological functions normally associated with growing old are manifestations of aging at the molecular level. The long-range goal of research in this area is a broad base of basic data which can be applied to solution of clinical problems of the elderly.

The projects currently being supported in this area fall into these general categories: the effect of aging on enzymes and enzyme systems; the effect of aging on chromosome structure; the effect of aging on the synthesis and degradation of macromolecules and small molecules; age associated changes in the structure and function of connective tissue; the effect of aging on the structure and function of biological membranes; aging in model biological systems.

Research obtained within these projects over the past year suggest that both the concentration and activity of several enzymes undergo characteristic changes in aging experimental animals.

In another area of active research investigators are studying the changes which occur in connective tissue, especially collagen, as a result of aging. In particular, research is underway to examine the synthesis and degradation of collagen as well as the type and position of intermolecular cross-linking.

A new area, in which research was initiated only during the past year, is the effect of age on chromosome structure. Studies are underway to examine age-related changes in the association of histones with DNA.

The possible role of membrane deterioration during aging is also being examined. The calcium and phosphate transport systems of the mitochondrial membrane are being studied to determine if aging has any influence on their activity.

The effect of aging on the synthesis of mRNA is also being examined. Special attention is being given to RNA's containing a poly - (A) tract. Another aspect of protein biosynthesis being studied is the possible age associated accumulation of defective tRNA's.



Areas judged in need of further research activity include: age-related changes in the activity and molecular properties of regulatory enzymes; the effect of aging on the synthesis, degradation, and properties of lipoproteins and glycoproteins; age associated changes in cytoskeletal components such as microtubules and microfilaments; the effect of aging on the structure of chromatin; the role of tRNA isoacceptors in aging.

#### Diabetes in Aging

Although the high incidence of diabetes in the elderly has been observed for many years, the cause underlying this apparent phenomenon still remains unknown. The problem is a complex one. For instance, it is not clear whether the loss of glucose tolerance with age is true diabetes or simply a non-pathological manifestation of aging. If the latter is true, then new standards of comparison must be devised for use in testing the elderly for diabetes. In addition, there are other problems uniquely associated with the elderly diabetic. They are especially dependent on family, friends, and the community for help in management. Psychological factors such as depression may also influence the motivation of the elderly diabetic's self management.

Research support in this area should be broadly based and concerned with studies on the nature, epidemiology, etiology, pathogenesis, treatment, and complications of diabetes associated with the aging process.

Currently, projects are underway in the following areas: changes in the regulation of insulin production with aging; the relation between Beta cell insensitivity to glucose and tissue sensitivity to insulin; role of decreased binding in tissue insensitivity to insulin; comparison of in vivo and in vitro aging with respect to lipoprotein binding, uptake, and degradation in smooth muscle cells obtained from normal and diabetic donors.

Recent studies indicate that newly secreted immunoreactive insulin is heterogeneous with regard to molecular weight. Furthermore, it has been found that the molecular weight distribution of immunoreactive species elicited in response to glucose changes with aging. As many of the newly discovered species as possible are being purified and characterized and their biological function tested.

Another factor being studied is the effect of dietary management. The results of this study indicate that manipulation of the dietary intake of carbohydrate protein and fat may prove to be helpful in the prevention or amelioration of the glucose intolerance observed during aging.

Areas judged presently in need of more research activity are the following: social and psychological factors affecting the elderly diabetic; the influence of aging on the mechanism of insulin release; insulin biosynthesis and processing; suitable techniques for the culture of Beta cells.

#### PROGRAM DEVELOPMENT MEETINGS HELD DURING FISCAL YEAR 1978

##### Workshop on Prospects for Research in the Biochemistry of Aging

The purpose of the workshop was to evaluate the present state of research on the Biochemistry of Aging and to define new biochemical approaches to this area which requires further development. Topics discussed included: the mechanisms

underlying the progressive onset with age of glucose insensitivity exhibited by B cells; the nature and development of age-related insulin resistance in target tissue; the process of intra-cellular protein degradation and factors influencing protein turnover; the accumulation of particular enzymes in aging organisms; basic studies on the effect of aging on the composition and function of the pericellular matrix; and, the control of genetic expression with aging. The meeting was chaired by Dr. Richard C. Adelman; ECRP staff leadership was provided by Dr. Lester Smith

#### Federation of American Societies of Experimental Biology

In response to an invitation from FASEB, members of the Biological and Clinical Sciences sections of the Gerontological Society presented abstracts and participated in a series of seven Aging Symposia at the annual meeting of FASEB, April 9-14, 1978. Topics covered by the symposia included an Overview of the Biology of Aging, Disease and Aging, Nutrition and Aging, Pharmacology and Aging, Systems Physiology and Aging, Cell Biology and Aging, and Molecular Biology and Aging. NIA staff leadership was provided by Dr. Lester Smith; additional NIA staff participating included the Chiefs of the Basic Aging and Biophysiology and Pathobiology Programs, the Director of NIA and the Associate Director of ECRP.

#### Workshop on Pharmacology and Aging

In collaboration with NIGMS a workshop was held to consider approaches on the various physiological and pathological parameters which affect drug distribution, metabolism, pharmacokinetics, drug-drug interaction, factors which influence gastrointestinal absorption, animal models, and other topics of major importance. Those specific drugs addressed included tranquilizers, anti-hypertensives, anti-arrhythmics, and antibiotics. It was apparent from discussion that the problems of pharmacology and aging must be addressed on several different levels. In addition to physicians, other health personnel, including pharmacists and dieticians, must be alerted to the factors which are particularly relevant to geriatric patients. Finally, methods must be explored to enable the older patient himself to participate in the responsibility for appropriate use of therapeutic agents. A published report of the workshop appeared in Fiscal Year 1978 and is available upon request. Staff contact- Dr. Lester Smith.

#### Immunology Planning Workshop

This workshop convened July 27, 1978 to obtain the advice and the recommendations on the content and participation of a major Immunology Conference planned for fiscal year 1979. Those areas to be represented include: phylogeny and ontogeny of the immune response, cell senescence, immuno-endocrinology, tolerance and autoimmunity, histocompatibility systems and life span, immunoregulation, longitudinal studies, immunodeficiencies, amyloidosis, and various other immune dysfunctions related to Progeric subjects and Down's Syndrome. Approximately 40 participants, NIA grantees, and other immunologists will take part in a two day conference to be held at NIH, Bethesda. ECRP staff leadership - Dr. Lester Smith.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative Research Program

Contract Number: NIH-AG-14

Contract Title: A study of Lymphocyte Function and Serum Immunoglobulin Concentration During the Life Span of Individual Mice

Contractor: Cornell University, New York, New York  
(Contract Officer: Dr. Marc Weksler)

Money Allocated: \$62,883

**Objectives:**

To assess immune reactivity, by measuring the proliferative response of blood lymphocytes in culture and by measuring the plasma concentration of immunoglobulins. These immunological parameters will be correlated with longevity.

Although defects in immune function of aged mice have been repeatedly demonstrated, it has never been shown whether immune reactivity has a positive, negative or little survival value. That is, do animals with vigorous immune reactivity die early while those possessing lower reactivity survive or does immune reactivity decline with age and those with the most impaired immune function die first. Either hypothesis would result in the observed paired immune function observed in old mice.

**Results:**

Data obtained on 100 Balb/c, 111 C57Bl/6J and 100 CBF mice on a longitudinal basis have been obtained during the past 18 months. The data has been entered into a computer and the first outputs are now available covering the first 15 months of life with respect to changes in serum immunoglobulin concentration and PHA stimulated lymphocyte proliferation.

Immunoglobulin serum levels increase with age. During the first year of life the serum concentration of IgM increased 51-56%; of IgG<sub>1</sub> 32-39%; and of IgG<sub>2A</sub> 37% in the three strains studied. The increase did not result from a loss of animals with low serum immunoglobulin levels.

Proliferation with blood lymphocytes cultured with PHA declined with age in all three strains studied. The Balb/c and C57Bl/6J declined 53% over the first year of study. The F<sub>1</sub> mouse declined only 31%. This appears to result from a loss of lymphocyte reactivity with age and not a loss of high responders from the cohort study.

#### Significance to Aging Research:

The immune system is known to undergo a marked functional decline with advancing years. Evidence exists that immune dysfunction is to some extent involved in the pathogenesis of essentially all age-related diseases. We hope to ultimately understand more about the immune processes which mediate the onset of autoimmune wasting diseases, such as cardiovascular disease, vascular damage, kidney disease, rheumatoid arthritis, as well as those immunological processes which may contribute to the increased appearance of neoplasia.

#### Proposed Course:

The correlation of these changes with survival of individual mice and the onset of autoimmunity will be made during the next 18-24 months.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative Research Program

Contract Number:       NIH-AG-7-2108

Contract Title:        The Relation of Age to Adverse Drug Effects and  
                          Other Factors

Contractor:            Boston University Medical Center, Boston, Massachusetts  
                          (Contract Officer: Dr. Hershel Jick)

Money Allocated:       \$55,102

Objectives:

To develop information on the relationships of clinical drug effects to age. This was a retrospective study, the task of which was to organize existing data so as to identify and define patterns of practice of therapeutics in relation to age. The Boston Collaborative Drug Surveillance Program served as the data source.

Significance to aging research:

It is well recognized that preliminary testing of drugs in the laboratory or in animals is insufficient for full understanding of their effects in man. Careful observation of patients treated with drugs is a critical requirement for a more complete understanding of human pharmacology. There are, of course, a complete set of potentially interacting factors which may influence any individual's response to drugs. These include drug factors such as dose, route of administration, and duration of treatment. They also include patient factors, such as age, disease state, weight, liver and kidney function, and blood chemistries. Finally, drug effects may be influenced by the administration of other drugs. In view of this complexity a realistic understanding of human pharmacologic response required the acquisition of data on many factors. It is factors with clinical outcomes or events.

Results:

It is important to consider dose when evaluating the relation of drug toxicity to age, since this contract also showed a positive correlation between dose and toxicity. In general, it has been observed that attending physicians tend to give lower dosages of drugs to the elderly.



Other clinical correlates were also found:

- 1) There was a strong positive correlation between age and the level of BUN. Since the kidney plays a major role in the elimination of many drugs, this finding has substantial implications in the drug treatment of the elderly.
- 2) Levels of SGOT and Bilirubin did not correlate with age; levels of Alkaline Phosphatase tended to be elevated in the elderly. The absence of correlation with age of those tests which measure liver cell function suggests that the liver of the elderly may not be substantially impaired in terms of drug metabolizing activity.
- 3) There was a reasonable clear negative correlation between age and the level of serum cholesterol. This may be due to selection such that those who survive to reach old age tend to have relatively low serum cholesterol levels, though it is also possible that lower levels in the elderly may be a reflection of different dietary habits among people in the different age groups.
- 4) There is a progressive fall in serum albumin with increasing age. This has important implications for therapeutics, since many important drugs tend to be bound tightly to albumin.
- 5) There was a strong positive correlation between systolic pressure and age and no appreciable correlation with diastolic blood pressure and age.

Overall, there was no consistent relation between age and drug toxicity. For most drugs the adverse reaction rates in older patients are grossly similar to those in younger patients. This is the case despite the fact that older people tend to weigh less, have poorer renal function, and lower serum albumin levels. On the other hand, older patients tend to receive lower doses of drugs.

This contract examined in considerable detail the clinical toxicity of over 100 drugs in relation to age. With some exception, there was little correlation between age per se and toxicity. Exceptions are as follows:

- 1) Heparin - There appears to be a higher risk of bleeding, without a corresponding improvement in efficacy in women over the age of 60 who receive Heparin.
- 2) Diazepam and Chlordiazepoxide - Central nervous system (CNS) depression attributed to the benzodiazepines was more common with increasing age. CNS depression was indicated by drowsiness among non-smokers, light smokers (20 cigarettes a day or less), and heavy smokers who received Diazepam, Chlordiazepoxide, or

Phenobarbital for anxiety. The age trend was not evident in patients who received Phenobarbital.

- 3) Potassium Chloride - The most common adverse reaction attributable to Potassium Chloride was hyperkalemia, which occurred more frequently in elderly patients.
- 4) Flurazepam - Toxicity as measured by unwanted residual drowsiness increased with age progressively from 1.9% among those under 60 to 7.1% among those over 80 (P less than 0.001). Unwanted effects of high-dose Chlorazepam were observed much more commonly in the elderly. Low doses of chlorazepam appear not to result in unwanted problems.
- 5) Nitrazepam - Central nervous system depression was significantly more frequent in the elderly. The effect of age on the reported rate of unwanted CNS depression was more striking at high doses.
- 6) Digoxin - The clinical toxicity of Digoxin tends to be weakly correlated with age.

This contract reported the reexamination of data from the Boston Collaborative Drug Surveillance Program to evaluate whether there is an indication that certain drug interactions tend to be more common in the elderly. There is no substantial evidence that such is the case. The only interaction found was a weak association with age in the interaction between Potassium Chloride and Spironolactone in producing hyperkalemia.

Staff recommendation:

This contract has contributed to a better understanding of the pharmacology of several drugs consumed by the elderly. A study of this nature, however, results in associations and correlations which are of some value in themselves. But without physiological and clinical data on the patients suffering adverse reaction, the value of the data is limited. These data will serve as the basis for decisions to program specific research related to the clinical status of the individual experiencing adverse drug reactions.

BIOPHYSIOLOGY AND PATHOBIOLOGY  
AGING PROGRAM

The Biophysiology and Pathobiology Aging Program's (BPAP) primary emphasis is given to: 1) Nutrition and Metabolism, 2) Endocrinology and Physiology, 3) Neuroscience, and 4) Pathobiology and Development of Vertebrate Animal Models, particularly mammalian models. The first three are directly linked by their closely interrelated influence on regulation, control and modulation of body systems to maintain and support homeostasis, as well as structural and functional development of these systems. Because epidemiology and clinical investigations are closely related to these three areas of research, clinically oriented studies in ECRP are given primary focus in the two program areas of Research Geriatric Medicine and Epidemiology. Animal models are of central importance to the development of studies on how each system or process influences, controls, or changes with age. Animal models are used in the study of aging to replicate, simulate, extrapolate or contrast somparative aging processes in humans. The Animal Models program is not only a focal point for development of research within BPAP, it is also an important resource and research tool in the entire NIA.

Nutrition, Metabolism and Aging

The purpose of the research program on nutrition and aging, an increasingly important NIA component, is to establish the interrelationships between dietary intake, disease prevention and optimal health maintenance in the aged, as well as the influence of nutrition on basic processes of aging.

Clinical nutrition research in relation to aging processes, disease prevention and health status maintenance is in a relatively primitive state of development, as is evidenced by the almost complete lack of information or data on standards for the nutritional requirements of the elderly. These requirements and the utilization of nutrients are largely unknown and those employed are based primarily on extrapolations from younger age groups. The primary and secondary effects of dietary deficiencies, excess drugs, physiologic decrements in functional responses, environmental and psychologic stresses are complex and are further complicated by: 1) the lack of firm scientific data as a basis to sort out cause and effect relationships, 2) a paucity of clinical data as a basis for therapeutic intervention to treat disease and degenerative processes and 3) the lack of information to educate the aged on nutrition because even the basic nutrient requirements for older people are unknown, and their special needs untested.

Except for lipid metabolism and the effect of a few vitamins and trace minerals, virtually nothing can be documented about the influence of nutrition on disease prevention or vigor in the aging adult. A host of interactions of deficiencies and excesses in micronutrients such as vitamins, minerals, and drugs are known to be associated with central nervous system disturbances, to influence normal wound healing, affect metabolic processes and physiologic function. But, firm data to establish these relationships are missing. Many, if not most, of the debilitating conditions seen in the aging adult are related to a nutritional deficiency or excess. Until this information can be acquired and validated through basic and clinical nutrition research and



integrated into preventive medical practice, the health of the aging adult will continue to be compromised.

Basic nutritional research on aging has repeatedly demonstrated that caloric restriction can improve the health and longevity of laboratory animals. It extends the maintenance of near optimal physiologic function well beyond the norm, defers the onset of age-associated pathologies such as neoplasms, renal and cardiovascular disease, and usually increases longevity by about twofold. It is an established and important finding. But the underlying medicating mechanism is unknown. The effect of caloric restriction on immune response, endocrine and neuroendocrine function is under study on a very limited scale. The results thus far are tantalizing and suggestive. But, until this phenomena is tested longitudinally in mature and aged animals, its specificity for influencing aging processes and its application to human health is unknown. Therefore, unless broader based studies are mounted, it will remain an enticing but largely unexplored phenomena of great potential for the improvement of human health. NIA presently supports a few individual studies ranging from nutritional requirements of the aged through the effect of varying dietary intake on physiologic responses, and the development of age-associated pathologic processes.

It is clear that the adequacy of the level and balance of intake of essential nutrients provided by the diet affects the functional efficiency and morphologic structure of all organisms and probably the psychological responsiveness and social activity of the aged. Throughout life, the health status and response of the individual to the challenges of disease, environmental stress and aging depends upon the quality and quantity of nutrients consumed, their absorption and utilization, as well as the adequacy of current dietary intake. Thus, research in appropriate animal models and human population on nutritionally mediated influence on cellular morphology and function, endocrine response, metabolic processes, and immune function may provide some of the most significant answers needed to moderate or prevent the development of mental and physical disabilities that currently characterize progressive deterioration and susceptibility to disease that occurs as a function with age.

Current research supported by NIA aimed at providing some of the answers to the influence of nutrition on aging and aging processes is highlighted by several of the following examples:

Management of body weight poses a dilemma for the elderly. Obesity is closely associated and, in some cases, a contributing factor in many of the age-associated diseases. However, a common accompaniment of stress in the elderly is a precipitous and oftentimes irreversible decrease in lean body mass that leads to morbidity and mortality. Studies with animal models have examined the relationship between dietary intake and the development and maintenance of optimum body weight. It was shown that restriction of caloric intake increased longevity in rats, but without further increments in lean body mass. The stress-induced loss of body weight in old rats could be ameliorated, but the process resulted in greater than normal mineral requirements and was dependent on the quality as well as quantity of protein ingested. There was evidence that food restriction during development decreased the number of fat cells and altered the sites of deposition, as well as maintaining lipolytic hormone responsiveness at older ages. Other beneficial results from early and

constant food restriction were lower serum triglyceride levels and prevention of age-related increases in serum cholesterol and phospholipids.

A preliminary clinical study utilized measurements of obligatory urinary and fecal nitrogen and indicated that loss was the same in elderly as in young females and found no difference between ages in total protein requirements. Additional results also suggested that elderly men did not require higher protein intake than younger ones.

#### Program Development Efforts

In support of its goal to broaden the base of knowledge in clinical nutrition, the Institute sponsored a clinically oriented research conference at Bethesda, Maryland, on June 5, 6, and 7, 1978, chaired by Dr. Robert E. Shank, Washington University School of Medicine, St. Louis, Missouri. The aim of the conference was to establish the state of knowledge about clinical nutrition and aging and provide NIA with advice and guidance on development of its research programs in this area. The conference resulted in a series of recommendations for medical research, which provided the basis for an Announcement to the scientific community aimed at encouraging the development of studies on nutritional requirements, health status and wellbeing of the aged, as well as basic aging processes.

### ENDOCRINOLOGY, PHYSIOLOGY AND EXERCISE

#### Endocrinology

The Endocrinological research support focusses on studies that seek to determine the mechanisms responsible for reproductive senescence.

Seven project grants, 1 program project and 3 fellowships address reproductive endocrinology. All of these focus essentially on the alterations with age in the brain-hypothalamus-pituitary-gonadal axis. Changes with age are measured in behavioral, biochemical and physiological systems that directly or indirectly affect the reproductive process. One project is in clinical endocrinology, but the remainder involve aging rodents as model systems for the study of pre- and post-menopause phenomena. A variety of procedures are utilized to block or augment normal steroid, peptide and protein hormone synthesis and release. Hormones measured include neurotransmitters and releasing factors in the brain and hypothalamus, trophic hormones from the pituitary, and those hormones released from the various target organs that modulate peripheral physiological responses and in turn affect the hypothalamo-hypophyseal axis by feedback control. Other separate studies suggested that catecholaminergic mechanisms at the brain and hypothalamic level were involved in aging processes of the brain-pituitary-ovarian axis. Dietary inclusion of the catecholamine precursor, L-tyrosine, or direct placement of L-dopa into the medial preoptic area of the brain restored ovarian function in aged rats. In addition the brain of old rats remained sensitive to estrogens suggesting the ovary and not the CNS was the primary site of dysfunction. Results from still another study also showed that factors in the hypothalamus rather than in the ovaries were responsible for loss of estrus. It was again suggested that decreased catecholamines and increased serotonin concentrations in the hypothalamus were indicated. It was repeatedly confirmed that decreases in gonadal steroid hormone secretion resulted in elevations of gonadotrophic releasing hormones from the hypothalamus and rises in circulating gonadotrophic hormones from



the pituitary of old female rats. These resultant hormone imbalances also contribute to the anovulatory condition in menopause. A third factor is that an increase in extra ovarian conversion of androstenedione to estrone occurs in overweight females in the post-menopausal period. This process occurs in adipocytes; it is important because age and obesity have been associated with an increased probability of endometrial carcinoma. Many peripheral physiological responses are altered by these hormonal imbalances which retard the reproductive process. For example, menopause is associated with an increase in cystic follicles and proliferation of connective tissue; affinity of the uterus for steroids and morphology of the uterine linings change with age; peripheral vasodilation results in "hot flashes" and it is suggested that competition between catechol estrogens (formed from estrone) and catecholamines for the degradative enzyme catechol orthomethyl transferase may be the underlying cause. In the male, reproductive capacity does not always wane with age, but prostatitis is a universal problem. A study in dogs elucidated some of the steroid biochemistry associated with benign prostatic hyperplasia and showed that 3- androstenediol plus 17  $\beta$ -estradiol enhanced growth and that 3  $\alpha$ -hydroxysteroid dehydrogenase enzyme activity was elevated during accelerated prostate growth, and under androgen control. This animal model will continue to be utilized to study the mechanisms controlling prostatic growth.

In still another animal model study, investigators examined the hypothalamo-hypophyseal-adrenal-gonadal axis to determine if non-specific stress predisposes tissues and organs to premature vascular disease and aging. Initially it was shown that early castration prevented the increase in blood pressure that normally occurs with age in spontaneously hypertensive rats; accelerated hypertension, aging and vascular disease were also demonstrated in continuously breeding rats compared to intermittent breeders. The results suggested multiple hormonal interaction may influence genetic programming toward hypertension and aging.

In another approach, cell cultures established from embryonic chick ovaries provided an animal model for aging, since in the normal course of development the left ovary grows and the rudimentary right ovary ages rapidly and involutes. This system was utilized for an in vitro examination of hormone responses. The ovarian anatomy, estrogen production, and response to exogenous pituitary trophic hormones were all depressed in the aging ovary compared to the growing ovary.

Endocrine dysfunctions directly or indirectly are contributing factors in many of the disabilities of the elderly. Some prevalent disorders with associated endocrine dysfunctions that appear to deserve immediate and continued research in the elderly include: organic brain diseases: neuro-endocrine metabolism and function in brain and hypothalamus; thermoregulatory impairment: thyroid hormone, and adrenomedullary catechol amine metabolism and function in brain, smooth and skeletal muscle, and fat; osteoporosis fractures: parathyroid hormone, calcitonin, and thyroid hormone metabolism and function in blood and bone with associated vitamin and mineral nutrition; reproductive senescence: pre- and post-menopausal changes in women, and benign prostatic hypertrophy in men; diabetes: insulin glucagon, somatomedins, and adrenocortical steroid hormone metabolism and function in blood, liver, muscle and intestine.

## Physiology

Six projects examine the control of physiological systems, and alteration of these during aging. Specifically they are composed of studies on renal and liver function and biochemistry, thermoregulation, and theories of aging including control of basal metabolic rate and alterations in circadian rhythms with age.

One study of renal function demonstrated a deficiency of enzyme machinery in renal membranes, probably because of age-associated errors in protein synthesis. There were increases in glomerular permeability and proteinuria with age that overloaded mesangial phagocytic function, and old rats were also unable to combat acidosis because of failure of compensatory enzyme adaptation.

Another investigation looked at circadian rhythms, specifically examining changes in circadian organization with age and the question of physiological penalties incurred as a result. Organisms with multioscillator systems can have their internal timing disrupted by driving at different system frequencies. The free-running period of slave rhythms in real circadian systems were generally well away from the central pacemaker period, and this was close to 24 hours. The closer together these two became the more sensitive they tended to be subject to internal temporal disorder.

Among areas which may be developed in the future is the study of systems physiology, so necessary to the definition and delineation of disease processes and aging processes; such an area would include investigations of the nervous, circulatory, respiratory, digestive, excretory, and musculoskeletal systems in the elderly. Since the separate processes in disease and aging are both comprised of multifunctional responses to internal and external change (where coordination of the whole organism takes precedence over individual organ and cell responsiveness) a thorough understanding of physiologic function in systems of the aged organism must necessarily underlie investigations of disease in those systems. The long range purpose, is of course, to provide knowledge on which we can base logical courses of prevention and treatment.

## Exercise

Support for work in exercise physiology consists at present only of a single research training grant in human physiology and a research grant in exercise physiology utilizing animal models.

The research training grant, clinically focussed, deals with environmental stress and adaptation, thermoregulation, cardiovascular adaptation, and respiratory responses to exercise in male and female subjects

The research grant supports basic animal work aimed at development of a scientific basis for the intelligent use of exercise as a tool in preventive and therapeutic medicine. The study identifies and describes the major adaptive changes induced by exercise in the skeletal muscle, cardiovascular system and body composition which will result in increases in functional capacity. In fundamental research on the mechanisms responsible for the increase in work capacity during chronic exercise and for the development of muscle fatigue during acute exercise it was found that chronic exercise induced a biochemical adaptation which results in a slower rate of carbohydrate utilization in muscles of trained animals. These adaptations included

feedback inhibition of glycogenolysis by substrate inhibition of phosphorylase<sub>b</sub> activity, and direct inhibition of glycogenolysis by free fatty acids.

Evidence was developed showing that elevated free fatty acids increased the capacity for prolonged running in rats by their glycogen-sparing effect and that glycogen depletion was an important cause of fatigue during prolonged exercise.

Further, the research showed that the time course for adaptation to endurance exercise was very rapid in the rat heart, with a 31% increase in weight and a half-time of about 5 days, and a loss of adaptation or regression of cardiac hypertrophy occurring 21 days after cessation of training.

Finally, cold exposure or administration of thyroid hormones mimicked the increases in muscle mitochondria that occur during endurance exercise, indicating the same factors were responsible in both cases, e.g. accelerated ATP hydrolysis and ADP formation.

Some critical areas in exercise physiology and aging needing further research have already been identified: the delineation of optimum exercises for maintenance of mobility and flexibility of joints and muscles in the elderly; determination of the nature and breadth of psychological benefits of exercise for the elderly; determination of what constitutes adaptation for the elderly and what role it plays in preventive and rehabilitative medicine; studies to examine the effects of acute versus chronic exercise in the elderly; integrative studies that address the importance of nutrition in exercise, and neuroendocrine and endocrine modifications during exercise. It is anticipated that future program development efforts in the area will be directed toward these, doubtless along with other issues yet to be identified.

#### Pathobiology and Development of Animal Vertebrate Models

The development of practically every field of biomedical research depends on relevant and appropriate laboratory animals as research models. This is particularly true for studies on basic biologic, physiologic, pathologic, and psychologic processes. The basic function of most endocrine organs was defined by studies on laboratory animals. Screening for carcinogens and cancer chemotherapy has been largely accomplished in genetically defined and characterized mice and rats. Initial studies on most drugs, such as today's antibiotics, were carried out in animals before their introduction into man. Our knowledge of immune response and, in particular, the decline in immune function with age, has been significantly enhanced by studies in animals free of known pathogens. The requirements for laboratory animals for research on aging are the same as for other areas of study, but with the additional stringent requirement that significant numbers of animals must survive to old age.

Animals to be used in aging studies must be maintained as nearly independent of disease and environmental insult as is consistent with the natural progression of aging processes over time. Late-occurring age specific, pathologic and degenerative changes must be anticipated and controlled for in establishing the baseline biological characteristics of the many animal species and strains over their life span. This imposes a heavy financial and logistical



burden in research on aging, involving even short-lived animals such as rats and mice because: 1) large standing colonies must be planned and maintained due to the need for reserve cohorts of aging animals and increased mortality with advancing age, 2) environmental control must be rigidly established and maintained, 3) extensive life span characterization and actuarial tables must be developed and periodically redefined, 4) a wide variety of animal models must be knowledgeably selected, characterized, and developed, since aging processes differ among and between animal species, including humans. Animal species other than rodents are needed to study various aging processes within and between species.

Gerontological research in mammalian models, and quite likely in all vertebrate species, is probably unique with respect to the scientific and technical judgments that must be exercised in the selection, development, and rearing of animal models for research on aging. Aging studies increasingly require animals defined by genetic background, biological and behavioral characteristics, control of environmental status, and current actuarial data for the strain and species under study. The information and data that describes these characteristics of the strain or species beyond reproductive age, necessary to the selection of a species or strain for research on aging, is scarce and scattered through a wide variety of scientific and technical publications and laboratory records. The acquisition and evaluation of the quality and significance of this information and data is not usually within the capability of the individual or group of investigators at a single institution. The space requirements for rigid environmental control for periods in excess of three to five years is usually not available for chronic studies or is outside the capabilities of many university-based animal programs. The set aside of animals to age and acquire age specific degenerative, pathologic and survival information and data cannot usually be accomplished by the individual investigator for each study of a strain or species because costs would be prohibitive. Therefore, the major responsibility for planning the development, characterization and availability of species and strains of animals as models for the study of aging must be assumed by NIA because of the responsibility for encouraging the development of research on the biological, behavioral and social processes of aging.

A major contribution to the development of animal models for gerontological research is the development and availability of aged mice and rats for use in research projects supported by NIA and pilot studies that may suggest formal studies on aging. The development of selected strains and stocks of laboratory mice and rats was initiated in March 1970 by a pilot study to establish the criteria for selection of rodents to study aging and the environmental conditions necessary for control of pathogenic micro-organisms, parasite, and environmental stresses of temperature and humidity fluctuations, light-cycles, noise and methods of handling.

This project over the past eight years has supported collaborative research projects to establish: the feasibility of developing and providing pathogen free aged rats and mice from a central breeding laboratory; criteria necessary to rear aged animals independent of infectious disease; that aged animals free of pathogens can be shipped by commercial carrier to laboratories throughout the USA; the age specific characteristic of selected strains of aged,

inbred rats and mice including pathologic and age associated morphologic and functional changes; actuarial data basic to the planning of studies in aging animals; a program of allocation and distribution of aged mouse and rat strains from a central resource colony; a reporting of data and information on the strain developed for research on aging.

Currently, the National Institute on Aging maintains under commercial contract a colony of Fischer 344 rats (male and female) and C57BL/6 (male and female), BALB/c (male), and CBF/1 (male), (a hybrid cross of BALB/c X C57BL/6) inbred mice. Aged rats and mice, 3 to 30 months of age are provided to investigators for pilot studies in anticipation of later submission of a research grant application, as well as for predoctoral candidates whose studies will form the basis for a dissertation on aging research. During the past three years, this program has provided 7,872 aged mice and 10,967 aged rats in support of over 168 projects on aging research. This includes support of extramural and intramural projects, research grants, contract and pilot studies, as well as the availability of biological materials to ancillary projects.

The rapid pace with which aging research has developed and the increased sophistication of these studies requires a wide variety of animal models. There will be continuing need to identify, select, define, and make available to meet the increasing need for relevant and appropriate animal models to study and compare aging processes. In anticipation of the increased amount, specificity and refinement of research on aging, several programs to support these requirements have been put in place by NIA. These include: 1) Development of a standing colony of eleven genotypes of mice: C57BL/6; BALB/c; DBA2; B6D2F<sub>1</sub>; CBA/ca; 129; Nude (Swiss-Webster); C57BL/6/bg/bg; CBF<sub>1</sub>; CBA/HT6; B6C3F<sub>1</sub>. 2) Selection and development of an F<sub>1</sub> hybrid aging rat genotype from among three inbred hybrids: F344 X Brown Norway (BN); F344 X Lewis (LEW); F344 X Buffalo (BUF). The aim of this project includes development of several between strain hybrids using both albino and pigmented strains and evaluation of the relative merit of each hybrid, selection of one or more hybrids for development and availability in aging research. The hybrid should minimize the disadvantages of an inbred strain, permit retention of genetic control, provide greater uniformity in phenotypic characteristics and evaluate the comparative utility of albino versus pigmented rat hybrids. 3) Commissioning the development of a taskforce to assess "relevant and appropriate animal models for research on aging" aimed initially at evaluating and characterizing selected small laboratory animals and nonhuman primates: a) Rats; b) Mice; c) Lagomorphs; d) Gerbils and Guinea Pigs; e) Carnivores f) Nonhuman Primates. 4) Survey of available aged nonhuman primates and identification of animals that may be set aside fifteen-plus years for research on aging: a) nonhuman primates in national primate centers; b) tag-on studies to on-going research projects, i.e., heart, cancer and drug studies. 5) Planning the development and availability of nonhuman primates and feline models for the study of aging. The aim of NIA's program on animal models for gerontological research over the next several years is to: a) consider all current animal models used in aging research and establish their relevance and/or appropriateness as models of aging processes and/or mechanisms in man; b) find out the kinds of models that may be needed in aging research; c) acquire data and information on models that may be more informative than current models; d) develop criteria for the selection and development of models for aging research; e) determine conditions under which genetic or environmental variability (disease, nutrition, etc.) in animal models will interfere



with interpretation of studies in aging; f) develop methods and approaches to, availability of animal models or biological materials for research on aging. Clearly, the identification, selection, development and characterization of a wide variety of species and strains of animals is basic to the advancement of aging research.

#### Research Geriatric Medicine and Epidemiology

Clinical investigation in geriatric medicine and its base in epidemiology are consolidated in this report to represent NIA's extramural research in both these areas. This program is aimed at the development and support of clinical research on the medical problems of the elderly that may influence maintenance of health, quality of medical care, establishment of health care requirements, medical practice, diagnostic, surgical, and preventative procedures.

The relative and absolute increasingly larger populations into older age cohorts requires the development of research programs on medical problems unique to the aged adult. Studies are needed to define the current basis for medical care provided to the elderly. These studies are needed for the development of medical care provided to the elderly that will prevent or moderate, rather than exacerbate long standing chronic impairments associated with aging. Basic to these studies is the epidemiologic methodology necessary to identify medical, physical, and psychological risk factors in the aged and weigh the relative risks and benefits of therapeutic and nontherapeutic interventions in the aged patient.

The current emphasis of this program element is aimed at studies that deal primarily with studies on the clinical and medical problems of the aged. One NIA-supported research program examined biological, social and psychological factors impinging on the nervous system that alter functional responsiveness and result in normal aging processes. Evidence emerged, that early morbidity and mortality was genetically determined; it was suggested that lower levels of immune and central nervous system (CNS) function were causal factors. Predictors of higher survival rates included low cholesterol, cardiovascular health, and high social activity, while CNS defects were a predictor of low survival rates. Some of the results suggest a uniform cognitive decline with age, which was general in males, but specific in females for a decline in processing of non-verbal stimuli. One of the aging processes that may be involved in cognitive decline was shown to be a marked reduction in regional cerebral blood flow correlated with decreased vascular reactivity to carbon dioxide. The location of the impairment was found to be the same as the focal neurological defect demonstrated.

Research geriatric medicine using epidemiology as a tool so far has not made significant progress toward defining norms for aging in the way that pediatrics has established standard norms for the development of children. Therefore, new areas for future development should include building toward physiologic norms that take into account the functional and morphologic consequences of age and their implications for medical and clinical care of the elderly. This will necessitate clinical studies on the interrelationship of environmentally related conditions, pathophysiologic processes, age associated diseases and their clinical and medical implications. Already identified needs for clinical investigation in geriatric medicine are: the implications and interrelationships of cardiovascular, renal, respiratory, gastrointestinal, muscle

skeletal, nervous system changes with age and the expression of clinical symptomatology and functional response; pathophysiologic changes that may influence medical and clinical care such as menopause, benign prostatic hypertrophy, osteoporosis, hypertension, and susceptibility to hypothermia and hyperthermia; environmental influence related to accidents, injuries, and falls in the elderly; the interrelationship of infections, disease, trauma and surgery to the special medical problems and care of the aged; nutrition and its relationship to obesity, glucose tolerance, lean body mass, susceptibility to disease, wound healing, and general health status of the aged, particularly the influence of chronic nutritional excess and deficits on expression of age-related disease in the aged adult.

## Neuroscience of Aging

Functional relationships between the aging process and correlated changes in the central nervous system (CNS) are of central importance. The principal goal of this portion of the BPAP is to expand the pool of knowledge, in both basic and clinical neuroscience, as related to aging; such development is essential in an effort toward practicle clinical solutions to infirmities associated with progressively deteriorating neurological functions in the elderly.

One recent activity to this end was a Research Workshop on Sleep and Aging held on June 1 and 2, 1978. Staff organizer was Dr. Betty H. Pickett, NIA's Associate Director for ECRP. The Workshop was Chaired by Dr. William Dement, Stanford University. The purposes of the Workshop were to take an overview of the state of knowledge on sleep as related to the problems and processes associated with aging and to generate recommendations to NIA for needed research and research resources in this field. The nineteen conferees included numerous active sleep researchers, plus several scientists from the fields of neuropsychology, physiology, and gerontology not now actively working in sleep. The conferees included four sleep researchers currently supported by NIA. The report of the Workshop and its recommendations is in process of preparation now; it is anticipated that dissemination will take place early in the succeeding fiscal year. The subject of sleep research will be handled subsequently by Dr. Zaven Khachaturian of the NIA's Biophysiology and Pathobiology Aging Program. He will be the Health Scientist Administrator responsible for further development.

Several outstanding projects in both fundamental and clinical neuroscience are supported by NIA. A representative sample of these and recent findings can illustrate the common thread of knowledge which runs through them.

It is well known that neuronal loss occurs with age in the brain. However, it is not known whether or not cell loss results in axon sprouting and the reorganization of existing circuitry. An understanding of the reorganizational capacity of the aged brain might provide insights into possible underlying changes in mental capacity. A current study investigates the capacity of the senescent brain to support axon sprouting following partial denervation.

The dentate gyrus of the hippocampal formation was used as a model system and the response of afferents following unilateral lesions studied in 3 month old and 24 month old rats. In young animals removal of the entorhinal input to granule cells elicits reorganization characterized by an outgrowth of the commissural-associational fiber plexus, increased positive staining of AChE, and repopulation of the dendritic field as seen by ultrastructural and analysis. Aged animals respond to such a lesion with similar circuitry changes but the magnitude of the changes are less dramatic. The outgrowth of the commissural-associational fiber plexus is on the average reduced, AChE positive staining is less pronounced and the dendrite field does not appear to repopulate to the same degree as in your younger animals over a period of at least 2 months. Curiously, although some animals show robust plasticity others show almost none.

These findings correlate with findings from behavioral studies which also indicate that old animals are a non-uniform population, showing variable performance. It may be that inherent plasticity properties of the senescent nervous system correlate with the decline in mental function.



It has been suggested that deficiencies in brain vasculature might be one of the underlying causes for the loss of brain function with age; examination of the vasculature in the hippocampus of adult and aged rats has yielded provocative findings.

The general pattern is similar in both groups but in older animals the vessels have a distinctive appearance. The vasculature in the hippocampus of senescent rat shows a definite decline in the delicate appearance of the vessels, as compared to the structure in young animals; in old rats appearance of the vasculature no longer demonstrates the same degree of fineness and divergence as in the younger animals. The old animals vessels now appear somewhat swollen, and their orientation tends towards a horizontal rather than a perpendicular direction. This pattern is strikingly similar to that observed in brains of young rats which have suffered partial brain damage.

The hippocampus and the adrenocortical hormonal system are believed to exert major reciprocal influences upon one another. In another set of experiments, hippocampal changes in relation to plasma levels of corticosterone and aldosterone was studied as a function of age. In this study an anatomical measure was used to assess degree of brain aging because it appeared to be a stable measure. It was found that levels of the adrenocortical hormones and weight of the adrenal gland change with age. These changes appear to be correlated with the degree of brain aging. These hormonal changes could be causative to brain aging; could be a consequence of brain aging; or could be independent, and a consequence of a 3rd factor influencing both variables.

There is growing scientific interest in the possibility that brain aging may act as a "pacemaker" to other forms of aging, particularly through neuro-endocrine deregulation. This possibility is emphasized by the similarity, noted by several investigators, of the mammalian aging syndrome to the syndrome associated with pathological elevated adrenal hormones (Cushing's syndrome).

If the present hypotheses prove to be correct, these studies might lead almost directly to health benefits. That is, therapies against the most deleterious aspects of brain and somatic aging might be developed which would depend upon manipulation of plasma hormonal levels.

Another area of interesting investigation deals with the effect of aging on brain monoamine-containing neurones and the relationship of monoamines to neurologic and endocrine changes. It has been shown that there is a selective impairment during aging in dopamine level in the striatum, median eminence of the hypothalamus and in the neural lobe of the pituitary.

Although Parkinson's disease is established as a disease of dopamine deficiency, the extent to which dopamine functions are impaired during normal aging has remained unknown. Recently, two reports from Europe confirmed that there is a progressive loss of striatal dopamine in normal aging humans which parallels with findings in animals.

Another project involving human brains ranging from 15 to 60 years of age has also shown that, of all the enzymes studied, those involved in the synthesis

of dopamine (tyrosine hydroxylase and DOPA decarboxylase) exhibit the greatest reduction in activities with age. These changes are reported to be most striking in the substantia nigra, but also in the caudate and putamen, and to a lesser degree in all other regions measured.

#### Future Plans for Program Development

The general objectives of the Neuroscience Program will be implemented by encouraging both fundamental and clinical research in four general areas of Neuroscience.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: N01-AG-7-2127

Contract Title: Aging Retired Breeder Sprague-Dawley Rats

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$48,000,00

- Objective:
1. To establish a ready resource of commonly used laboratory rats on which base line nutrition, biochemical, and physiologic data is already available.
  2. To provide a genotype rat that may be used for comparison and evaluation of the generalization of data across and between species.

Significance for Aging Research: The study of aging processes requires the availability of a wide variety of strains and species of animals, as well as resource materials from aged animals. Stocks and strains, such as the Sprague-Dawley, on which vast amounts of baseline biochemical and functional measures have been developed, must be provided for research on rats. Established developmental parameters in these animals can then be compared with late occurring aging processes. Well characterized stocks used in basic biomedical research are the starting point for such studies. Therefore, availability of this commonly used stock makes an important contribution to the development of research on aging.

Proposed Course: This colony will be maintained, primarily self-sustaining, for at least two years.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: N01-AG-4-2811

Contract Title: Development of a Production Colony of Three Genotypes of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$149,025

- Objectives:
1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.
  2. Develop a ready commercial source of aging mice of three basic genotypes to meet the demands for aging laboratory mice.
  3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.
  4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.
  5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency, studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability, a colony of aging mice of the inbred strains C57BL/6 BALB/c, the inbred F<sub>1</sub> hybrid of the two inbred strains, was established in a barrier enclosure (SPF)<sup>1</sup> at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes proposed under this research contract provides investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This has moderated one of the primary constraining influences on the development of aging research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific genetic control, 2) for study, one of several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status.

Proposed Course: Contract will continue for a minimum of two years with the contract becoming increasingly self-sustaining in subsequent years as animals are provided to investigators for research on aging.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Program

Contract Number: N01-AG-6-2135

Contract Title: Selection and Development of an F<sub>1</sub> Hybrid Aging Rat Strain

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$73,000.00

- Objectives:
1. Development of a rat model for aging research that provides a broad gene pool and maximum genetic control.
  2. Develop an F<sub>1</sub> hybrid strain that avoids pathologic lesions of body systems and endocrine tumors that are seen in most inbred strains and outbred stocks of rats currently available.
  3. Provide a strain of rat for research on aging that is less susceptible to environmental change than are in the currently available inbred strains.
  4. Provide a rat strain which has much of the generalizability to aging as an outbred stock with uniformity and predictability of pathologic and biologic characteristics of the inbred strains.
  5. Characterize the major biologic and pathologic changes over the life span of the strains selected for development.
  6. Select from among three between-strains crosses with the Fischer 344 the F<sub>1</sub> hybrid cross representative of the characteristics believed to be of greatest general applicability to the studies of aging in the rat.

Significance for Aging Research: Studies utilizing animal model systems are unique with respect to the considerations that must be exercised in the development of aging animals. Current and projected experiments in aging will require animals of defined genetic background, known biological characteristics and environmental status. Only with meticulous and exacting control of the many interacting genetic, physiologic, pathologic and environmental variables will it be possible to develop relevant animal models that may explain many of the biologic processes in aging. The F<sub>1</sub> hybrid rat model is necessary in aging research in view of the need for a rat model that is genetically defined, characterized biologically and represents a broad gene pool that permits generalizations of findings to the species as a whole.

Proposed Course: Study completed, for selection of  $F_1$  Hybrid January 1980.  
Development of  $F_1$  Hybrid strain should commence in FY 1980.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: N01-AG-7-2128

Contract Title: Development of a Colony of Multigenotypic Mouse Strains

Contractor: Charles River Breeding Laboratory, Wilmington, Massachusetts

Money Allocated: \$201,763.00

Objectives: 1. To develop, define and make available the primary strains of laboratory mice that are needed to provide model systems of maximum flexibility and applicability necessary for increasingly sophisticated aging studies in biomedical research on nutrition, genetics, immunology, biochemistry, physiology and psychology.

Basic to a program of studies on comparative aging is the development and availability of a variety of species and strains of aged laboratory animals that can be used as model systems for the study of aging processes. To facilitate this program, defined and controlled animal model systems must be developed that will meet the needs of investigators in aging research. The eleven genotypes of aged mice developed under this project will enhance the ability of investigators in aging to study, trace and compare the differential aspects of aging processes by using unique attributes of mouse strains with chromosomal markers, identifying cellular characteristics, differential genetic traits and age specific morphologic and functional difference between strains and between strains F<sub>1</sub> hybrids. Thus, this project will enhance the quality and quantity of aging research and moderate a major barrier to research on aging by making available basic mouse strains: C57BL/6, BALB/c, DBA/2, B6D2F<sub>1</sub>, CBA/ca, CBF<sub>1</sub>, C57BL6bg/bg, for research on aging processes.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: N01-AG-7-2118

Contract Title: Assessment of Relevant and Appropriate Models to Study  
Aging Processes

Contractor: National Academy of Sciences, National Research Council,  
Institute of Laboratory Animal Resources

Money Allocated: \$198,000.00 (through FY 1979)

Objectives: The primary objective of this project is to acquire, evaluate, develop, interpret and make available information and data on basic criteria necessary for the selection of vertebrate models to study aging processes.

The selection of relevant and appropriate animal models in the study of aging processes is complete. Selection criteria are needed to aid investigators in choosing species wisely. Unfortunately, selection and evaluation criteria for vertebrate models of aging are not currently available. Relevant information is scattered in the scientific literature and technical publications, unpublished laboratory records, and the personal commentaries of investigators experienced with particular species are additional sources of information. Therefore, the task to be accomplished by this project is to assemble and critically review relevant information on the selection of vertebrate models for research on aging, and to preface a comprehensive report containing basic criteria for their use and limitations in research on aging.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Program

Contract Number: N01-AG-5-2854

Contract Title: Aging Monkey Tissues and Organ Resource

Contractor: Washington State University, Pullman, Washington

Money Allocated: \$6000.00 (FY 1978) continued from FY 1975

- Objectives:
1. To acquire organs and tissues from a rare resource of three (3) aged rhesus monkeys age 24 to 26 years as the animals become moribund or expire.
  2. To select and preserve organs and tissues from each of the animals that are or may be required for the study and inter-species comparison of aging and aged changes in the Rhesus monkey and other mammalian species.
  3. To bank fresh, frozen or chemically fixed and preserved tissues and organs from each of the three monkeys as they become moribund or expire.
  4. To provide selected tissues and organs on request for studies in aging.

Significance for Aging Research: The study of aging requires the availability of tissues and organs from a wide variety of strains and species of animals. To study aging changes and the comparative differences between the ordered life-span of different species of mammals requires that the program identify and develop resources that meet the needs of the investigator in aging research. Preservation and provision of tissues and organs from aged subhuman primates, essentially expiring from natural causes, will provide a continuing resource of rare and unique materials that would otherwise be lost to aging research. The contract essentially support the complete postmortem evaluation and preservation of tissues and organs of each of the three monkeys as they become moribund and/or expire. Postmortem protocol will require that all tissues and organs be examined, classified, and characterized. Tissues and organs from all major body systems and the integument will be selectively preserved based primarily on the requirement of the individual investigators. Other tissues and organs will be preserved by freezing or fixed in chemical as well as preparation of slide sets of tissues from major organ systems. These materials can be provided on request for study of aging changes in the subhuman primate or comparative studies between species.

Proposed Course: Contract is to be continued for a minimum of two years.

NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: N01-AG-3-2725

Contract Title: Maintenance of a Long-Term Aged Rat Colony

Contractor: Charles River Breeding Labs, Inc.

Money Allocated: \$375,000

The purpose of the contract is to provide rats suitable for research on aging to investigators funded by the NIA. NIA requires the contractor to acquire, maintain and ship male and female Fischer 344 rats that have been reared on a defined diet and specific pathogen-free conditions. Characterization of the physiology and pathology of this rat strain have been accomplished and made available to investigators in two publications (refs). The maximum life span was 30 months with 95% survival. The major age-onset pathology is glomerulonephrosclerosis which occurs in 85% of the rats at 24 months.

The colony thus provided disease-free rats of either sex to facilitate research on normative aging processes or the complications introduced by known pathogens.

The colony census currently totals around 9000 animals from 1-30 months of age and is being expanded by the monthly addition of 650 one month old virgin males, 75 one month old virgin females, and 150-250 retired breeder male rats. Pathological monitoring is continuing on a reduced basis. Predictions of optimum addition and deletion rates for the colony based on past, present and future investigator usage, are being facilitated by computerization.

Diversification of rat production among individual investigators would be cost prohibitive to them and cost inefficient for the NIA. The steady budgetary increases of the NIA provided both the explanation and the justification for expansion of the animal resources. The rational development of a biological research program in aging requires a parallel, if not a greater rate of expansion of animal resources for investigation.

Future needs: The size of the animal colony must be adjusted to adequately meet the demands of an expanded base of biological research in aging. Predictions of need will be obtained by computer-assisted analysis of data and examination of the history of growth and utilization of animals by the NIA.

Proposed course: Contract will be supported for a minimum of three more years and will become increasingly self-sustaining.



NIA Annual Report  
October 1, 1977 through September 30, 1978

Extramural and Collaborative  
Research Programs

Contract Number: NO1-AG-6-2136

Contract Title: Aging Barrier Sprague-Dawley Rat Colony

Contractor: Harlan Industries Incorporated, Indianapolis, Indiana

Money Allocated: \$102,710.00

- Objectives:
1. To meet the current demand for a commonly used stock of laboratory rat.
  2. To develop a commercial resource of aged virgin male rats on a defined diet in an essentially pathogen-free environment for investigators in aging research.
  3. To provide a ready resource of aged rats that may be used to develop techniques and procedures for studies on aging as well as pilot studies for the acquisition of preliminary data as a basis for determining feasibility for research on aging.

Significance for Aging Research: One of the primary barriers to the development of a program of studies in aging research is the lack of availability of laboratory animals in varying degrees of senescence that are representative of the aging process. To facilitate the development of aging research, standardized strains, stocks and species of animals that can be used as simulation models of aging processes must be developed in quantities that meet the needs of investigators in aging research. Since a single strain, such as the Fischer 344 cannot serve as a model for all studies, a commonly used rat of different genetic characteristics is required for a significant number of studies in aging. The Sprague-Dawley represents a distinctly different rat model for the study of aging.

Proposed Course: Feasibility of continuation will be under study for a minimum of six months.

## BEHAVIORAL AND SOCIAL SCIENCES AGING PROGRAM

The NIA's Congressional mandate calls for support not only of biomedical research, but also of social and behavioral research and research training on aging. At the present time, about 25% of the Institute's Extramural and Collaborative Research Program funds are expended in these latter two areas, and the outlook for the future is for intensified efforts by the NIA to expand support for research which will lead to increased knowledge concerning changes in behavioral function with age, and how individuals and societal institutions cope with and adapt to such changes.

The following sections report on present foci of NIA support in these areas, highlight recent findings and indicate areas where further development is required.

### BEHAVIORAL RESEARCH ON AGING

#### Cognition and Intellectual Function and Age

A sizeable portion of the behavioral research supported by NIA is concerned with the identification and specification of cognitive and intellectual changes that occur with aging. Most of this work is done with humans, although some of the studies deal with age-related decline in memory ability in rodents. The potential utility of such research is at least twofold: to describe fundamental changes in the normal organism with age; to form a baseline for the diagnosis and therapy of pathological deficits in cognition such as are found in senile dementia; to provide information of value in setting retirement policies, and for designing work and other aspects of the environment so as not to penalize older people.

Several of the currently funded projects study the processes of human learning and memory acquisition retention and recollection of various materials which are usually verbal, (although interesting work is being done with memory for pictures or spatial displays and for the interaction between verbal and spatial materials in acquisition, retention and recall). All the studies supported tend to find that learning is slower for old humans than young, regardless of kind of learning materials involved. Researchers find that older learners, in contrast to younger, apparently do not take full advantage of opportunities to group or relate the materials to be learned for most efficient encoding and memory retention; nor are older people as able as the young to take advantage of instructions on how to do so, and the age differences in this kind of performance tend to increase with increasing demands of the tasks. As a rule, the old learners tend not to be much poorer than the young in recognition ability of previously learned material of acquired items, but they are usually considerably poorer at recall.

In the foregoing work, as well as in other projects which compare cognition function in older and younger people for visual and spatial materials, the research investigators do not discuss "memory" in isolation but regard learning, retention and recollection (or "acquisition processes," retention and "retrieval processes") as three aspects of a single task. A tentative conclusion of such work is that, with discrete, meaningful items to be committed to memory, most of the age-differences are at the input end, with most older subjects studied being less than most younger subjects to organize or "encode" the memory materials for efficient storage and recollection.

In experiments aimed at testing function of the two cerebral hemispheres, a short word is shown to viewers, either in the left half only, or right half only, of his visual field, so that the word is transmitted only to the right cerebral hemisphere or only to the left. In most people of all ages, verbal materials received by the left hemisphere only are more easily perceived than are verbal materials received by the right hemisphere only. This difference is found to increase with adult age, which may indicate that either right hemisphere functions decline more with age than left hemisphere functions, or else that the two hemispheres become increasingly unable to perform each other's usual functions. Further current work is testing the latter possibility.

Three projects, two of them only recently begun, are concerned with age-related changes in semantic memory, which refers to remembering the meaning of connected statements or discourse, in which the subject appropriately recalls his own verbal "encoding" or interpretation of the verbal stimuli. With subjects aged 19 through 69 years, no age differences were found (immediately after acquisition) in ability to integrate meaning from different sentences into wholistic ideas. However, the older subjects recalled only about 60% as much semantic information after a lapse of time, even though liberal paraphrasing was allowed in scoring the response. Further, recognition of materials was somewhat poorer for older subjects than young. The investigators found also, that providing contextual information in various ways made the tasks easier for all ages but did not diminish the age differences found. The researchers concluded that age differences in ability to comprehend abstract sentences were more important than memory per se in producing the decline with age in the accuracy of stored information. In work for which support has only recently begun, attempts will be made to develop ways of testing and measuring the semantic memory abilities of senile dementia patients.

A serious problem in the study of human aging, readily applicable to the study of cognition, is the extent to which differences between different age groups measured at one point in time represent intrinsic age changes rather than differences caused by possible differences in the life-histories of the different age groups (cohorts). Until this decade, it was assumed that the sizeable differences found between age groups in intellectual test performances reflected mostly age changes or intrinsic aging effects. But more recently, one investigator, who has conducted a complex, longitudinal study of changes in measures of primary mental abilities with adult age, concluded that most of the age-group differences obtained in his own studies represented historical or secular differences between successive cohorts rather than changes associated with aging, leading him to suspect that the decline with adult age was a "myth." However, more recent and further analysis of the same data by this investigator and by others have cast doubts upon the earlier conclusion. The matter is still controversial, but at this point, it appears that for some intellectual abilities, there is not much age decline until the early sixties, although there may be more decline earlier for some other important intellectual abilities. But final, firm conclusions cannot be drawn yet, and further work is indicated.

More applied approaches toward measurement of intellectual capacity are taken in other NIA-supported projects. For example, morbidity, rather than age per se is known to account for some apparent age-decline in cognitive performances,



in that retrospective analyses of performance scores in elderly groups usually show average performance differences in favor of those who survived over those who died within some period after testing. One study supported by NIA tested a large group of older subjects on a variety of cognitive and other skills and factors, with the intent of predicting survival. Preliminary analysis indicates that test scores and other non-medical measures may allow a very considerable increase over chance in the prediction of individual deaths. This emphasizes that "years before death" may be more important than years after birth in accounting for cognitive performance levels in older people.

Most psychological tests and assessments of possible chronic brain syndrome have been developed using institutionalized patients. Taking a different approach, NIA-supported investigators have commenced efforts to develop tests and measures for the study of non-institutionalized older people, among whom various degrees of chronic brain dysfunction are prevalent. Health, social support systems and other factors will be taken into account in developing tests and measures of actual functional capacities and chances for successful independent living with varying degrees of family and community aid.

Until recently, chronological age has been the primary criterion in assessment of work-related competency or in determining a time of retirement. Attention is now turning to the possibility of functional assessment of competency. Another group of NIA-supported investigators is attempting to develop a set of measures particularly suitable for older adults which will permit prediction of competent behavior in specific classes of situations. Such results will be particularly useful and relevant to non-chronological retirement criteria, or to issues such as competence to maintain independent functioning requirements for institutional support. As noted below, this is an area of particular interest.

#### Sensory and Perceptual Processes and Aging

NIA currently supports only few projects on the aging of sensory and perceptual function; these can be highlighted briefly as follows.

One group of current studies focussed on time required for processing complex visual information; is analyzing the nature of the differences between old and young individuals in ability to respond to a variety of brief, visually presented stimuli. Another NIA-supported investigator is making a careful analysis of age-related change in smell and taste sensitivity, a topic of great importance in relation to dietary intake and adequate nutrition of older people.

Animal research in memory function is covered in an earlier section of this Annual Report.

#### Social Science Research on Aging

NIA currently supports social science studies of aging across a range of topics, involving sociocultural and demographic approaches, research on the elderly family, issues related to retirement, and related matters

Study of the social and cultural context in which aging occurs, appears to require taking both a retrospective and a prospective view of the aging



process in which we can examine the various transitions and responses to them experienced through the life course; integration of knowledge of both individual behavioral and social processes appears necessary for an understanding of adaptation to aging. NIA supports a range of social science-oriented research including studies on community residents, institutionalized elderly, coping and adaptation patterns and attitudinal research. Longitudinal approaches are useful for obtaining information on significant social and cultural factors in aging overtime, for instance, on matters such as overall adaptation, sources of stress and coping responses. In one NIA-supported project, major life transitions and crises within the last few years in an aging population and the responses to these changes were studied. Respondents widely reported changes in one's parents as stressful events, and were more likely to seek outside assistance for problems with their elderly parents than for all other events, (except their own health), despite their attempts to obtain help, the study respondents faced with parental debilitation frequently reported high levels of stress. The relation between parent's health problems and respondent's poor adaptation increased with age. On the other hand, the departure of one's children from home, another common event in mid-life, was not associated with psychological stress. Respondents whose last child had left home during the last four years were characterized by high levels of satisfaction with their marriages, high life-happiness and low symptom levels.

Another study looks at middle-aged children's perceptions of their aged parents and relationships to them. Recent findings show that with the mental and physical decline of the parent, the type of care by offspring, often progressed to total care; helping to care for an elderly parent was considered to entail a sacrifice and was found to be related to lower morale. The data indicate that despite a strong sense of obligation, the burdensome aspects of the relationship tended to outweigh moral and emotional gratifications as the parent's dependence increased. Men showed a greater ability to withdraw emotionally and physically from a mentally deteriorating parent than did women, and it was the psychologically less robust and more dependent women who became preoccupied and experienced severe emotional stress when confronting the decline of the parent.

Some NIA support is provided for research on stresses associated with the adaptation to institutionalization of older people. Findings from a current study show that the stress of institutionalization can be decreased by providing relevant suitable and adequate information about the institutional environment. Nursing home residents who were provided with such information, were found (both in terms of nurses-ratings and self-ratings) to be healthier, better adjusted and more active in the nursing home than those who were not. Further results of the study indicate that self-assessed levels of self-esteem and feelings of control are good predictors of activity level and health status within the institution.

Only little NIA-supported research activity is currently focussed on the severe stress of bereavement so frequently experienced by and among older people. In an effort to encourage additional research applications on this topic, the NIA issued a program announcement on this topic as outlined below under Program Development Activities. A major substantive focus of the announcement was on the increased risk of morbidity and mortality associated with bereavement.

The increasing numbers of older people in the population present a new phenomenon for contemporary society. While demographic studies have focussed on projecting numbers of future elderly, there has not been much stress on the distinctive aspects of elderly migration. With the occurrence of retirement, some aging persons are free to move about the country yet we do not know much overall about their migration patterns. One current NIA-supported project deals with the determinants, distributional patterns and the outcomes of primary migration streams for those over 60 years of age during the period 1965-1970. Using a 15% Public Use Sample from the 1970 Census, a state-to-state origin-destination matrix for elderly inter-state migrants was constructed. The results to date seem to indicate differences between the population in general and the elderly group, as concerns both destination or origins of each "salient flow."

Another NIA-supported study of the aging population takes a historical perspective, analyzing social conditions of older Americans in the late nineteenth and early twentieth centuries. Through the statistical analysis of a large nationwide population sample for 1880 and 1900 supplemented by a special study of southern blacks in 1880, the investigators have looked at a number of issues, including family structure. They have reported for older southern whites the most distinctive family structure of any major group within the older American population; white southerners were most likely to live in extended families and to live in proximity to persons of the same surname (presumed kin). On the other hand, southern blacks, were much more likely to live apart from kin. Presently, there has been a shift among those over 65 years of age from living with, to living near their children. Yet, unlike the situation in 1880 and 1900, older blacks with children are more likely to live with them than are whites.

Other research areas in which demographic information is currently being obtained include socioeconomic determinants of mortality, sex differences in morbidity among the aging, and factors associated with mortality in widowhood. Of the nine primarily demographic studies now under way, a number have only just begun and have not yet gathered reportable findings. Subsequent Annual Reports will cover these projects.

#### The Family

Although NIA currently supports a few studies in the area, research on the aged and their families has been designated of special programmatic interest to the Institute; topics for development include the social networks of the elderly, with special reference to the significant social relationships of aging persons and how these change over time. Among the research questions regarding the relationships of aging to family interaction and social networks in general which should be explored extensively are demographic, social, psychological, economic, housing and other issues related to the elderly and their families, and family stability. The effort launched in this fiscal year through the National Academy of Sciences to establish a Committee on Aging (supported through an NIA research contract) has considerable potential for development of new research on at least some of these issues. See below for brief account of this activity.

## Retirement

Retirement is another area planned for special programmatic emphasis by NIA in the immediate future; to date relatively little such research has been supported by NIA. Among pertinent questions for exploration are factors influencing the retirement decision, impact of retirement on health and vice-a-versa factors related to successful and unsuccessful retirement. Research is also needed on the impact of voluntary and mandatory retirement, special issues concerned with women's retirement and the differential impact of retirement for minorities. A key question, addressed in the prior section on research on cognitive and intellectual functions, has to do with measures of functional age and functional competence, vis-a-vis retirement requirements.

## Program Development Efforts

### 1. Research Workshop on Older Women: Continuities and Discontinuities

Jointly sponsored by the National Institute on Aging and the National Institute on Mental Health, a Workshop on the Older Woman was scheduled for September 14, 15, and 16, 1978 in Bethesda. NIA staff responsibility was taken by the Associate Director, ECRP, Dr. Betty H. Pickett, with Mrs. Joyce Lazar, Chief, Social Science Section, Behavioral Sciences Research Branch, representing NIMH. The purpose of the meeting was to produce a set of research recommendations to the two Institutes for needed research concerning psychological and social aspects of the functioning of older women. Some 30 conferees were to be brought together to discuss a variety of topics focussed on the social system of the lives of older women and the effects of the presence of older women on the society. Topics to be discussed included health and mental health, health and Medicare, work and labor force participation, economic maintenance, Social Security, tensions, alimony and inheritance laws, social networks, family roles, loneliness and bereavement, illness and institutionalization, new roles and relationships, sex and intimacy, cross cultural and minority issues. A report of the Workshop recommendations will be prepared and distributed during the succeeding reporting period. The conference was to have been chaired by Dr. Helena Lopata of Loyola University, Chicago, but because of unforeseen circumstances was chaired by Dr. Pickett and Mrs. Lazar.

### 2. Research Conference on Memory and Aging

Sponsored by a conference grant from NIA, a research conference on memory and aging was held on August 11, 12, and 13, 1978 in Boston. NIA staff responsibility was carried by Dr. Walter Spieth, and the conference agenda consisted of four major sessions: a) encoding, storage and retrieval b) memory stores c) testing and intervention (clinical memory testing of aged patients) and d) new directions. Participants were of three types: senior researcher, prominent scientists not now working in the field of aging who have methodological ideas to contribute, and those who might be recruited into work on problems of aging. The Conference was Chaired by Dr. James Fozard of the Veterans Administration Out-patient Clinic in Boston.

Program Announcements on two topics in the Behavioral and Social Sciences were issued in the NIH Guide during Fiscal Year 1978, one on Retirement and Aging addressing the retirement issues outlined in the preceding sections and



·encouraging the submission of relevant applications. Similarly, an announcement concerned with Aging and Bereavement was issued at the same time. The hope is, of course, that through this and other mechanisms additional investigators will be encouraged to design and submit proposals for the support of research in these important areas.



NIA ANNUAL REPORT  
October 1, 1977 through September 30, 1978  
Extramural and Collaborative Research Program

Contract Number: N01-AG-8-2111

Contract Title: Committee on Aging

Contractor: National Academy of Sciences Assembly of Behavioral and  
Social Sciences

Principal Investigator: Sara Kiesler, Ph.D.

Money Allocated: \$186,700

Total for 2 Years: \$373,400

Objectives:

To assemble a Committee on Aging (consisting of 12 to 15 highly qualified behavioral and social scientists drawing from a broad array of disciplines such as sociology, social anthropology, demographic, economics, political sciences, etc.) which will function over a two-year period. The membership of the Committee is to include some behavioral and social scientists who have not previously conducted research on aging as well as some who have. The Committee will organize and report two research workshops, one focussed on the elderly family and the other on the "new elderly."

The major purpose of the activities of the Committee (which will include preplanning efforts such as preparation of appropriate papers, mini-conferences and consultations, etc.) are to interest additional highly qualified behavioral and social scientists in aging, to encourage additional attention to topics and approaches to behavioral problems in aging which may not have been fully researched to date. It is anticipated that the Committee's activities will generate recommendations to NIA for needed new research on the behavioral and social sciences of aging.

The Committee held its first meeting on May 2 and 3, 1978 and in the ensuing months, has developed articulated plans for the conduct of scientific meetings in fiscal year 1979 on the Aging Family, on the Elderly of the Future; the interface issues between biological and behavioral factors which affect the aging population are of special interest. The membership of Committee on Aging is broad, includes some established scholars in gerontology and a larger number of behavioral and social scientists who have not previously worked in the area. Disciplines ranging from psychology to economics, political science, demography and social work, are represented among the membership, as well as neuro-psychology and biology.



NIA ANNUAL REPORT

Report of the Gerontology Research Center

	<u>Page</u>
1. Office of the Scientific Director	GRC/OSD-1
2. Clinical Physiology Branch	GRC/CPB-17
3. Laboratory of Behavioral Sciences	GRC/LBS-110
4. Laboratory of Cellular and Molecular Biology	GRC/LCMB-168
5. Laboratory of Molecular Aging	GRC/LMA-208
6. Laboratory of Neurosciences	GRC/LNS-236





NIA ANNUAL REPORT  
October 1, 1977 through September 30, 1978  
GERONTOLOGY RESEARCH CENTER

Notable evidence of the Gerontology Research Center's emergence as the free-standing intramural program of the NIA was the inaugural meeting of its Board of Scientific Counselors in December 1977. Under the capable and enthusiastic chairmanship of Yale Professor Byron Waksman, the Board of eight outstanding scientists from the nation's academic community used its initial meeting to familiarize members with the GRC's organization, current research activities, and plans for new research initiatives.

Meeting again in May 1978, the Board undertook a searching analysis of individual research projects associated with the Baltimore Longitudinal Study. Their deliberations convinced them of the overall efficacy and value of the longitudinal method of doing research on human aging. In sum, the Board concluded that only longitudinal analysis can fully explore changes occurring in the same individual as he or she ages. Furthermore, longitudinal data must be obtained in order to validate information derived from cross-sectional investigations of age effects.

This year, the Center reorganized and initiated several research programs in an effort to expand the scope of its multidisciplinary investigations into the biomedical and behavioral factors associated with aging.

One major event was the introduction of females into the Baltimore Longitudinal Study of Aging. The addition of women in January 1978 offers scientists the opportunity to explore a variety of sex differences associated with the aging process in humans. The women's study design parallels that for the men with the main focus being on the description of physiological and psychological changes which occur over time. By the end of this reporting period, more than 100 women had made their initial visit.

The Center's research capability was enhanced with the establishment of the Laboratory of Neurosciences under the guidance of former NIMH senior investigator, Dr. Stanley I. Rapoport. This new laboratory investigates the central and peripheral nervous system and muscle in relation to changes in health, disease, and aging. Initial work is focusing on blood-brain barrier and central nervous system function; peripheral nerve and muscle function; and the pharmacology of the central and peripheral catecholaminergic nervous system.

Establishment of the Laboratory of Cellular and Molecular Biology brings together various research projects relating to the genetic basis for biological aging. Several programs from the former Laboratory of Cellular and Comparative Physiology and the Laboratory of Molecular Aging comprise this laboratory. Immunology research is now incorporated into the Clinical Physiology Branch where investigators are doing a comprehensive survey of immunological factors in longitudinal volunteers.

The NIA's first Professor Emeritus, Dr. James T. Irving, arrived this

year. An eminent scientist in the field of calcium metabolism and the processes involved in bone mineralization, Dr. Irving is attempting to develop a tissue culture model system for the study of osteoporosis.

The smooth operation of the Center this year was in no small part attributable to the excellent assistance provided by its internal committees. Invaluable suggestions and guidance came from many staff members who served on the Animal Resources, EEO, Library and Safety Committees.

#### Office of the Director

#### Section on Comparative Nutrition

This year, two studies carried out with Fisher 344 male rats showed a 35% and 47% decrease in the specific activity of liver aldolase, based upon the protein content of isolated cytosols. However, a smaller but statistically age dependent decrease (13%) was seen when Sprague-Dawley male rats were examined. Therefore, there are statistically significant decreases with age in the specific activity of this enzyme in rat liver although the decreases are smaller than those thus far reported in the mouse (>50%).

Recent data shows that feeding a low protein-high carbohydrate diet results in an increased enzymatic activity from 44% to 208% in mice. On the other hand, similar studies in rats indicate that enzymatic activity may actually decrease 42% while maximal induction was less than 50%. Similarly, the induction of aldolase brought about by feeding a fat-free high fructose-24% casein diet ranges from 20-28% in rats to from 90-112% in mice. It appears, therefore, that rats are less responsive to liver aldolase induction under a variety of dietary manipulations than are mice.

The age decrement in aldolase specific activity of liver cytosol in mice is reported to be associated with a change in its specific activity determined immunologically. In the coming year, biochemical studies are planned in which aldolase from young and old animals (rats and mice) will be purified by column chromatography and characterized by gel electrophoresis and isoelectric focusing. First, investigators will try to establish that the specific activity of purified aldolase is indeed low in the old animal preparations. Then, old mice will be fed ad libitum a fat-free high fructose-24% casein diet; reported here to indicate aldolase by approximately 100%. The induced enzyme will be purified and characterized in order to provide information as to whether age-associated inactive enzymes are the result of transcriptional, translational, or post-translational changes.

In another study, mean enzymatic activities per unit DNA of C57BL/6J female mice fed a 4% protein diet were markedly lower than those seen in animals fed a 24% protein diet. In the same study, other animal groups were fed the 24% protein diet intermittently (diet offered for 24 hours on Monday and Wednesday, and for 8 hours on Friday). The mean enzymatic activities per unit DNA of intermittently fasted animals were similar to those of the 24% ad libitum fed controls rather than to animals fed the 4%

protein diet. These data did not suggest the existence of a common biochemical alteration to explain the phenomenon of increased life span due to low dietary protein or intermittent feeding.

Marked variations in cellular enzymatic activities of animals subjected to intermittent feeding occur during the 48-hour interval of fasting and refeeding. Data for the present study were obtained at only two points during this time interval, and thus do not necessarily represent integrated cellular enzymatic activity. Final proof that these dietary restrictions reduce tissue protein synthesis awaits radioisotopic studies on animals subjected to various dietary manipulations.

From research examining the aged digestive system (esophagus, stomach, duodenum, jejunum, ileum and colon) by SEM, it appears that villi in the duodenum become more narrow with increasing age. By comparison, the duodenal villi of mice placed on dietary restriction appear longer than those of control animals of the same age (12 months). The greatest effect is seen in the low protein (4%) ad libitum group. Kidney glomeruli of the 4% protein group are significantly smaller than controls. Glomeruli from intermittently fed mice are not significantly different in size from the controls.

The size of liver cell bodies and liver cell nuclei are unchanged with dietary restriction. The TEM and SEM studies of normal aging animal tissues will continue. The SEM examination of the aging digestive system should be completed by the end of the calendar year 1978.

IMR-90 cell lines (population doublings 18, 30, and 49) were examined for structural factors which are altered with age. Results showed that with increased population doublings, a larger percentage of cells have numerous microvilli. By TEM, IMR-90 cells appear similar to the WI-38 cell line. Dense bodies are often of the ringed membranous type rather than the granular visiculated type seen in many tissues. Mitochondria, granular endoplasmic reticulum, and other organelles are present in large amounts.

Studies being initiated in the coming year will investigate the effect of aging on vitamin absorption and gastrointestinal transit time. Young, adult and senescent Wistar rats will be used. For vitamin absorption studies, rats will receive labeled doses of selected vitamins via stomach tube. At predetermined times following intubation, the gastrointestinal tract, blood, and several tissues will be analyzed for content of the administered vitamin. To determine transit time, non-absorbable aqueous and lipid phase markers will be administered. Structural differences in the gastrointestinal tracts of young, adult, and senescent rats also will be determined using the electron microscope.

#### Technical Development Section

The Technical Development Section has continued to provide maintenance and development services to the scientific programs of the Center. Several new instruments have been developed.



Every program at the Center received support from the Section's Mechanical Shop. Examples include: hemispherical rat exercisers for the Psychophysiology Section; cell harvesters for the Clinical Immunology Section; a muscle chamber for laser diffraction studies in the Cardiovascular Section; and an EM tissue processor for the Comparative Nutrition Section. Additionally, shop personnel rekeyed over 300 locks at GRC.

A second computer system to more than double our present capacity will be operational soon. With the addition of two tape drives, the system will provide staff with increased disc space, memory size, tape speed, and computational capability.

Microprocessor computer systems were implemented to meet instrumentation needs of the GRC. This relatively new technique allows moderately coupled instrumentation to be designed and fabricated quickly. The following systems were completed and installed:

1) Fragillograph data reducer--Inorganic Biochemistry Section; (2) Strength Data Acquisition System--Inorganic Biochemistry Section; (3) Spectrophotometer Baseline Computer--Inorganic Biochemistry Section; (4) Complex Concept Learning, and a Choice Reaction Time Test--Learning and Problem Solving Section.

Other systems expected to become operational in the coming year include the following; (1) Tapping Test--Human Performance Section; (2) Heart Rate Histogrammer--Psychophysiology Section; and (3) EM Tissue Processor--Comparative Nutrition Section.

Finally, a system of transferring data from the laboratory microprocessor systems to the main computer system, via programmable read-only memories, was developed.

### Animal Resources Facility

This past year the Animal Resources Facility was fully accredited by the American Association for Accreditation of Laboratory Animal Care. Modern caging and automatic watering systems were installed throughout the ARF providing more efficient care of animals.

In the open colony, care and housing were provided for approximately 90 rabbits (1500 received and issued during the year), 700 rats (600 received and issued), 12,000 mice (10,000 received and issued), 43 beagles (ages 3 to 17 years), 12 rhesus monkeys, 10 squirrel monkeys, 6 chickens and 1 goat.

The oldest animals in the C57BL/6J mouse colony are nearing 24 months of age and will be issued in the coming year. The population totals over 4,000 mice and is expected to reach 12,000 within the next year.

The closed aging colony of outbred Wistar rats is being increased in size from 10,000 to approximately 14,000 rats. There were 6,600 weanling rats introduced into the colony this year. A total of 3,089 rats were issued: 1,502 were 1-11 months old, 784 were 12-23 months and 803 were 24 months or older.



## Photographic and Arts Section

The Photographic and Arts Section supported the scientific staff by providing 665 illustrations, 5269 photographic prints, 3647 slides, and 14 posters for national and international meetings and publications. In addition, a new photographic print processor was acquired which should greatly increase our print production efficiency and capacity.

Among other projects completed this year, the section helped plan and produce materials for the Tachistoscope study and the study of distributive practice and free recall for the Learning and Problem Solving Section. For the Psychophysiology Section, extensive Photo-Macro techniques were used to produce photos of rat brain sections for an article on age related changes in the nigrostriatum.

In cooperation with the Information Officer, a photo display entitled "New Faces" was initiated. This display, in the main lobby, acquaints present staff with new employees. Photographs of all professionals were taken for a NIA Board of Scientific Counselors directory of the GRC staff.

## Information Office Activities

This year, the GRC Information Officer planned and coordinated the second NIA Science Writer's Seminar on Aging held in San Francisco. Two seminar papers are being prepared as additions to the science writer booklet series. A popular booklet on the Baltimore Longitudinal Study was completed and is scheduled for fall publication.

Some 400 visitors took part in 63 Center Tours. Visitors included heads of international institutes with aging programs, and scientists/clinicians from Britain, Canada, Chile, Japan, Poland, Russia and Scotland. Also, the Mayor of Baltimore, the Ad Hoc Coalition of National Organizations on Aging, National Council of Senior Citizens, NIA Board of Scientific Counselors, National Academy of Sciences staff, and new members of the National Advisory Council on Aging. Medical school or aging agency visitors came from California, Colorado, Maryland, New York, North Carolina, Pennsylvania and Washington, D.C.

Numerous print media contacts generated stories in the Chicago Tribune, Richmond Times-Dispatch, Washington Star, Baltimore Sun, San Francisco Chronicle, 73 Gannett papers, Harper's Bazaar, San Francisco Examiner, and the Kansas City Star, to name a few. This office arranged for "The Golden Age", a Utah monthly, to publish the nine booklet science writer series.

Center footage was shown on WBAL-TV, WBFF-TV, WMPB-TV, (all Baltimore channels) and WBBM-TV News (Chicago). Staff were heard over the Physician's Radio Network; the American Chemical Society's syndicated program, "Men and Molecules", KALW-FM (San Francisco); WAMU-FM (Washington); and on National Public Radio. WQED-TV (Pittsburgh) filmed for a documentary on aging; and filming was planned for KQED-TV's nationally shown "Over Easy".

Specialized media served included Ageing International, Older Americans Report, Geriatrics (three viewpoint interviews), Aging, NCOA publications, Retirement Living, JAMA, International Medical News, Medical World News, MD Magazine, Nightletter for U.S. Medicine, and PHS reports.

The Information Officer participated in the Baltimore City Fair, Towson State and University of Maryland-Baltimore County minimester aging courses, a Harford Community College Science Horizon's lecture series, the University of Maryland Journalism Careers Day, and spoke to the Highlandtown Kiwanis. Other activities included the continued internal publication of GERON-NEWS, coordination of annual and other recurring reports, work on the library committee, and orientations for new employees.

### Library Services

This year the Center Library handled over 1,200 requests for books and journal articles from the GRC staff. These references were obtained from the Welch Library, University of Maryland Health Sciences Library, National Library of Medicine, and NIH, among others.

Approximately, 4,900 titles were indexed for publication in the Journal of Gerontology under the title "Current Publications in Gerontology and Geriatrics". In August, the Library began using a computer terminal which will provide for on-line computer searches. Contracts signed with Medline and Bibliographic Research Services now provide the Library with access to several data banks containing over five million references.

The classification of the GRC collection continues. Early next year, the new catalog system will further help staff to locate books. Arrangements were made with the Welch Library to permit GRC scientists copying privileges, and an interlibrary loan service was established with the Library of Congress.

The GRC Library Committee helped determine periodicals to order or delete from the library collection. This committee also made recommendations to make more space available for the library collection. Staff provided increased services to other libraries, scientists, and students doing research on aging. Liaison with other gerontological centers was established whereby future exchanges of gerontology publications will be possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00101-02-SCN formerly Z01-AG-00101-01-LCP												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Relation between Nutritional State and Aging														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. H. Barrows</td> <td style="width: 20%;">Chief</td> <td style="width: 30%;">SCN, OSD, NIA</td> </tr> <tr> <td>Other:</td> <td>G. C. Kokkonen</td> <td>Chemist</td> <td>SCN, OSD, NIA</td> </tr> <tr> <td></td> <td>P. L. Mann</td> <td>Visiting Fellow</td> <td>CPB, NIA</td> </tr> </table>			PI:	C. H. Barrows	Chief	SCN, OSD, NIA	Other:	G. C. Kokkonen	Chemist	SCN, OSD, NIA		P. L. Mann	Visiting Fellow	CPB, NIA
PI:	C. H. Barrows	Chief	SCN, OSD, NIA											
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COOPERATING UNITS (if any)  None														
LAB/BRANCH Gerontology Research Center, Office of the Scientific Director														
SECTION Section on Comparative Nutrition														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland, 21224														
TOTAL MANYEARS: 2.75	PROFESSIONAL: 1.75	OTHER: 1.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) Contrary to published reports a statistically significant <u>age</u> decrease in the specific activity of aldolase in the liver cytosol of <u>rats</u> was observed. Rats appear to be less responsive than mice to the effects of age as well as to the induction of liver aldolase under a variety of <u>dietary manipulations</u> . The mean <u>enzymatic activities</u> per unit <u>DNA</u> of C57BL/6J female mice fed a 4% protein diet were markedly lower than those observed in animals fed a <u>24% protein diet</u> . In this study other groups of animals were fed the 24% protein diet <u>intermittently</u> (diet offered for twenty-four hours on Monday and Wednesday, and for eight hours on Friday). Those animals referred to as intermittent-fed were sacrificed either on Tuesdays or Thursdays. Those referred to as intermittent-fasted were sacrificed either on Wednesdays or Fridays. The mean enzymatic activities per unit DNA of intermittent fed and intermittent fasted animals were similar to that of the 24% protein ad libitum fed controls rather than to that of the animals fed the 4% protein diet. Therefore these data did not indicate the existence of a common biochemical alteration to explain the phenomenon of increased life span due to low dietary protein or intermittent feeding.														

Objectives: Attempts were made to establish: (1) whether aging is accompanied by the formation of selective errors in the genetic code which may occur with use; (2) whether dietary restriction which increases the life span of laboratory animals results in decreased tissue protein synthesis and a consequent decreased use of the genetic code.

Methods Employed: (1) The activities of aldolase and the protein contents of the cytosols were determined in the livers of rats (Fisher 344, Sprague-Dawley) and mice (C57BL/6J, CBA, CBAT6T6) of various ages fed either a diet which contained 24% protein, 4% protein, or a fat free-high fructose-24% casein diet. (2) C57BL/6J female mice were offered one of the following regimens: (a) a diet containing 4% protein fed ad libitum; (b) a diet containing 24% protein fed either ad libitum or; (c) intermittently (Monday, Wednesday and Friday). Those animals referred to as intermittent-fed were sacrificed either on Tuesdays or Thursdays, i.e. following a 24 hours feeding period. Those referred to as intermittent-fasted were sacrificed either on Wednesdays or Fridays, i.e. following a 24 hour fasting period. Succinoxidase, cholinesterase, malic dehydrogenase, DNA, and protein were determined by standard biochemical procedures.

Major Findings: (1) Two age studies carried out on Fisher 344 male rats indicated a 35 and 47% decrease in the specific activity of liver aldolase when based upon the protein content of the isolated cytosols. However a smaller but statistically significant age dependent decrease (13%) was observed when Sprague-Dawley male rats were examined. Therefore, there are statistically significant decreases with age in the specific activity of this enzyme in the liver of rats; however, the decreases are smaller than those thus far reported in the mouse (>50%).

Previous studies carried out in this laboratory have indicated that feeding a low protein-high carbohydrate diet resulted in an induction in the specific activity of liver aldolase of mice. Recent data have indicated that this dietary manipulation resulted in an increased enzymatic activity from 44 to 208% in mice. On the other hand, similar studies carried out in rats indicated that the enzymatic activity may actually decrease 42% while maximal induction was less than 50%. Similarly the induction of aldolase brought about by feeding a fat free-high fructose-24% casein diet ranged from 20-28% in rats but from 90 to 112% in mice. Therefore rats appear to be less responsive to the induction of liver aldolase under a variety of dietary manipulations as compared to mice. (2) In the study carried out on female C57BL/6J mice, differences in the concentration of DNA in the livers and kidneys indicated that fasting or feeding a 4% diet ad libitum resulted in small cells and that cells increased in size during a period of refeeding. In addition, the data indicated that a reduction in cell size was accompanied by reduced cellular protein. These changes in cellular protein were likewise approximated by changes in cellular enzymatic activities of succinoxidase, cholinesterase, and malic dehydrogenase. Calculated on the basis of DNA, the activities of these three enzymes were decreased in the 4% and intermittent-fasted animals and increased in the intermittent-fed animals. Furthermore, the mean values of the enzymatic activities per mg. of DNA of the intermittent-fed



and intermittent-fasted animals were essentially the same as that of the 24% ad libitum controls.

Significance to Biomedical Research and the Program of the Institute: (1) The aging concept proposed by Orgel based on random errors has been repeatedly refuted. However evidence is available which indicates that only selected enzymes are affected by aging. More importantly, this laboratory has shown that changes in these selective enzymes, when they occur, are found among the various species examined. These observations have led to the proposal that errors occur with age in the genetic code for these enzymes and the transcription of this information results in inactive enzymes.

The accumulation of inactive enzymes has been reported to occur in the aldolase of the livers of mice and rabbits but not rats. Since these reports seriously weaken and may negate the concept of selective errors, this laboratory initiated studies on the liver aldolase of rats. The results obtained during the past year clearly show that the specific activity of this enzyme in the rat is decreased with age and increased by certain dietary manipulations in a similar but less responsive way as observed in the mouse. (2) Although it is well known that dietary restriction increases life span of animals, the mechanism remains unknown. It was the aim of the study reported here to establish whether biochemical alterations were common in the cells of animals subjected to two dietary regimes reported to increase life span; namely, low protein and intermittent feeding. The mean enzymatic activities per unit of DNA of animals fed a 4% protein diet were markedly lower than those observed in animals fed a 24% protein diet. Therefore, these data support the hypothesis that dietary restriction increases life span by reducing protein synthesis and consequently reducing use of the genetic code. In contrast, however, the mean enzymatic activities per unit of DNA of intermittent-fed and intermittent-fasted animals were similar to that of the 24% protein ad libitum fed controls rather than to that of the animals fed the 4% protein diet. Therefore, these data, on the basis of enzymatic activity per unit of DNA: 1) did not support the hypothesis that dietary restriction, which increases life span, reduces protein synthesis and thereby reduces use of the genetic code, and 2) did not indicate the existence of a common biochemical alteration to explain the phenomenon of increased life span due to dietary restriction.

Proposed Course: 1) The age decrement in aldolase specific activity of liver cytosol of mice has been reported to be associated with a change in its specific activity determined immunologically. Biochemical studies will be carried out in which aldolase from young and old animals (rats and mice) will be purified by column chromatography and characterized by gel electrophoresis and isoelectric focusing. First, efforts will be made to establish that the specific activity of purified aldolase is indeed low in the preparation from old animals. Following this, old mice will be fed ad libitum a fat free-high fructose-24% casein diet which has been reported here to induce aldolase by approximately 100%. The induced enzyme will then be purified and characterized as previously described in order to provide information as to whether age-associated inactive enzymes are the result of transcriptional, translational, or post-translational changes. 2) Marked variations in the cellular enzymatic activities of the animals subjected to intermittent feeding occur

during the 48 hour interval of fasting and refeeding. The data of the present study were obtained at only two points during this time interval, and therefore do not necessarily represent the integrated cellular enzymatic activity. As final proof that dietary restriction reduces tissue protein synthesis, radioisotopic studies must be conducted on animals subjected to various dietary manipulations.

Publications:

Barrows, C. H. and Kokkonen, G. C.: The effect of various dietary restricted regimes on biochemical variables in the mouse. Growth 42: 71-83, 1978.

Barrows, C. H. and Kokkonen, G. C.: Relationship between Nutrition and Aging. In Draper, Harold, H. (Ed): Advances in Nutritional Research Vol 1 New York, Plenum Publishing Corp., 1977 pp. 253-298.

Barrows, C. H. and Kokkonen, G. C.: Comparative Nutrition During Aging. In Rechcigel, M. (Ed): Handbook of Nutrition and Food Cleveland, CRC Press, in press.

Barrows, C. H. and Kokkonen, G. C.: Diet and life extension in animal model systems. Metabolism in press.

Mann P. L.: The effect of various dietary restricted regimes on some immunological parameters of mice. Growth 42: 87-103, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00141-01-SCN												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  The Effect of Aging on Vitamin Absorption and Gastrointestinal Transit Time														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">C. H. Barrows</td> <td style="width: 30%;">Acting Chief, SCN</td> <td style="width: 20%;">OSD, NIA</td> </tr> <tr> <td>Other:</td> <td>B. B. Fleming</td> <td>Guest Worker</td> <td>SCN, OSD, NIA</td> </tr> <tr> <td></td> <td>J. E. Johnson, Jr.</td> <td>Staff Fellow</td> <td>SCN, OSD, NIA</td> </tr> </table>			PI:	C. H. Barrows	Acting Chief, SCN	OSD, NIA	Other:	B. B. Fleming	Guest Worker	SCN, OSD, NIA		J. E. Johnson, Jr.	Staff Fellow	SCN, OSD, NIA
PI:	C. H. Barrows	Acting Chief, SCN	OSD, NIA											
Other:	B. B. Fleming	Guest Worker	SCN, OSD, NIA											
	J. E. Johnson, Jr.	Staff Fellow	SCN, OSD, NIA											
COOPERATING UNITS (if any)  None														
LAB/BRANCH Gerontology Research Center, Office of the Scientific Director														
SECTION Section on Comparative Nutrition														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland, 21224														
TOTAL MANYEARS: 1.25	PROFESSIONAL: .25	OTHER: 1.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER														
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The effect of <u>aging</u> on the <u>absorption</u> of the fat soluble and water soluble <u>vitamins</u> will be determined in young, adult, and senescent rats. The <u>gastrointestinal transit times</u> of non-absorbable aqueous and lipid phase markers will be determined in the three age groups. <u>Structural changes</u> in the gut as a result of age will also be examined.														

GRC/OSD-11

Project Description:

Z01-AG-00141-01-SCN

Objectives: The objective of this project are to: 1. determine the effect of aging on absorption of a selected group of fat soluble and water soluble vitamins; 2. determine the effect of aging on gastrointestinal transit time; 3. examine structural changes in the gut as a result of aging.

Methods Employed: Young, adult, and senescent Wistar rats will be used in all studies. For the studies on vitamin absorption, rats will be given labeled doses of the selected vitamins via stomach tube. Animals will be sacrificed at predetermined times following intubation and the gastrointestinal tract, blood, and several tissues will be analyzed for content of the administered vitamin. A similar protocol will be used for determination of gastrointestinal transit time. For the purpose of determination of transit time, non-absorbable aqueous and lipid phase markers will be administered to the rats. Structural differences in the gastrointestinal tracts of young, adult, and senescent rats will also be determined by the use of the electron microscope.

Major Findings: The major studies are still in the preliminary stages. Extensive literature searches have been conducted and techniques to be used in the studies are being developed. A preliminary study to determine appropriate dosage levels and selection of a suitable lipid marker remain to be completed before the major absorption, transit time, and structural studies are begun.

Significance to Biomedical Research and the Program of the Institute: Research on the effect of aging on absorption and gastrointestinal transit time is very limited and inconclusive. An effect of aging on absorption may explain the observed reduced levels of various vitamins in the plasma of aged individuals and could result in altered recommendations for nutrient intake with potential for improved health in this group. Knowledge of the effect of aging on gastrointestinal absorption could also have important implications with respect to drug absorption. These studies should result in needed information on both structural and functional changes in the aging gastrointestinal tract. This information has practical clinical importance. In addition, this active system with its rapid cellular turnover may serve as a model system for the study of the effects of aging on function and structure.

Proposed Course: The effect of aging on the absorption of the fat soluble and water soluble vitamins will be determined in young, adult, and senescent rats. The gastrointestinal transit times of non-absorbable aqueous and lipid phase markers will be determined in the three age groups. Structural changes in the gut as a result of age will also be examined.

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBLR

Z01-AG-00142-01-SCN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Fine Structural Changes in Aging Cells and Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Johnson, Jr.	Staff Fellow	SCN, OSD, NIA
Other:	C. H. Barrows	Acting Chief, SCN	OSD, NIA
	E. L. Schneider	Medical Officer, PHS	LCMB, NIA
	R. G. Cutler	Research Chemist	LCMB, NIA
	E. Lakatta	Chief Cardiovascular Section	CPB, NIA

COOPERATING UNITS (if any)

J. Miquel, NASA Ames Research Center, Moffett Field, California  
M. P. Bear, Bedford, V.A. Hospital, Bedford, Massachusetts  
K. Nandy, Bedford, V.A. Hospital, Bedford, Massachusetts  
D. Brandes, Department of Pathology, Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center, Office of the Scientific Director

SECTION

Section on Comparative Nutrition

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland, 21224

TOTAL MANYEARS:

3.00

PROFESSIONAL:

1.00

OTHER:

2.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to examine, by electron microscopy, cells from tissues of mice, rats, monkeys and humans of various ages, to determine what similarities and differences exist, in terms of age related, structural changes, between different cell types of individual species and between similar cell types of the different species. Comparisons are being made between in-vivo models and in-vitro models at both the transmission and scanning electron microscopic levels. The effects of diet, stress, and drugs on the rate of aging at the cellular structural level are being examined.

GRC/OSD-13

Objectives: This research is oriented towards the study of cellular and tissue aging at the electron microscopic level. Anatomy is one of the most basic of the biomedical sciences and the electron microscope is our most powerful tool for study in this discipline. Very little is known about fine structural (electron microscopic) changes which occur with aging. This is true both at the transmission electron microscopic (TEM) level and at the scanning electron microscopic (SEM) level. Whole systems in the body are still almost unstudied with these techniques. Therefore, we wish to begin filling in the gaps in these unstudied systems with general baseline data and at the same time, search out the answers to questions involving specific intracellular activities such as the relationship of lysosomes, lipofuscin and mitochondria. We also wish to vary the environment, which, broadly, includes diet, stress and drug treatment, to determine if the rate of cellular structural aging can be manipulated.

Methods Employed: Mice of various ages ranging from 1 month to 40 months have been sacrificed by aldehyde perfusion. At present, these include untreated controls and also mice which have been on dietary restriction (intermittently fed or low protein diet, ad-lib). Since diet is one of the most powerful factors influencing life span, the dietary manipulation experiments are of great importance in this program. For comparison of in-vivo vs. in-vitro aging, human fetal lung fibroblasts and mouse neuroblastoma cells were fixed with an aldehyde combination in-situ and processed according to a new technique developed in our laboratory. Tissues from the perfused mice and cell cultures were prepared for TEM and SEM.

Major Findings: In examining the aging digestive system (esophagus, stomach, duodenum, jejunum, ileum, and colon) by SEM, it appears that villi in the duodenum become more narrow with increasing age. By comparison, the duodenal villi of mice placed on dietary restriction appear longer than those of control animals of the same age (12 months). The greatest effect was seen in the low protein (4%) ad-lib group. Kidney glomeruli of the 4% protein group were significantly smaller in size ( $\bar{x} = 58.5 \mu$ ) than controls ( $\bar{x} = 65.9 \mu$ ). Glomeruli from intermittently fed mice were not significantly different in size from the controls. The sizes of liver cell bodies and liver cell nuclei were unchanged with dietary restriction ( $\bar{x}$ 's for all groups were about  $28 \mu$  and  $8 \mu$  respectively). Glycogen content as studied by TEM varied in the restricted groups but no other major structural changes were noted. The in-vitro studies have begun to reveal a number of structural factors which are altered with age. The IMR-90 populations examined by SEM appear to have large numbers of cells which are very smooth on their surfaces (classified -). With increasing population doublings, a larger frequency of cells have microvilli on their surfaces (classified as +, ++, or +++ according to the extent to which the microvilli occur, with +++ cells having the most). In order to obtain numerical data, random areas were selected on the fixed and processed cell culture sample,  $10800 \mu^2$  in size (as observed on the SEM at 1000x). Individual cell regions  $108 \mu^2$  in size were then examined at 10000x. The cell surface was then classified as -, +, ++, or +++ as outlined above. Population doublings 18, 30 and 49 were examined. Percentage of the cell regions classified in the four categories

are shown below.

Classification	POPULATION DOUBLING		
	18	30	49
-	36%	20%	2%
+	54%	38%	46%
++	8%	38%	48%
+++	2%	4%	4%

It can be seen that, with increasing population doublings, more and more cells have numerous microvilli. (It was noted that cells at the tips of villi in the mouse intestine, ie., cells at the end of their life cycle, also have increased numbers of microvilli). Neuroblastoma cells by comparison, appeared to have numerous microvilli all the time; however, these cells are to be considered as a cancer cell rather than a normal cell type. Allowing the pH of the IMR-90 cultures to become on the acid side (about 6.8) resulted in a slowing down of cell division, and even young cultures (population doubling 18) exhibited numerous cells with large numbers of microvilli if kept in this acid medium. By TEM, IMR-90 cells appeared similar to the WI-38 cell line. Dense bodies were often of the ringed membranous type rather than the granular vesiculated type seen in a variety of tissues from perfused mice. Mitochondria, granular endoplasmic reticulum and other organelles were present in large amounts. Many cell were found to be degenerating if cultures became too thick suggesting that populations in cell culture laboratories should be kept under strict observation.

Significance to Biomedical Research and The Program of the Institute: The results, so far, on the nutrition studies indicate that various organ systems such as the kidney, liver and intestine are affected in different ways by dietary restriction in spite of a trend towards all the organs, and the animal as a whole, being generally decreased in size. The cellular reactions in dietary restricted animals, as seen with the electron microscope, may or may not fit into a general profile of slowed aging; this remains to be determined. The TEM examination of the IMR-90 cell line substantiates its choice as a replacement of the WI-38 cell line as the two lines appear similar. This is fortuitous, as examination of a third cell line, GM-1380, which is also a fetal lung fibroblast cell line, indicates that not all of the human fetal lung fibroblast cell lines are identical. The GM-1380 series has slightly different mitochondria than the IMR-90 and WI-38 lines indicating that some form of mutation may have occurred at some point in the early population doublings of the GM-1380 series. Thus, as new cell lines are developed for NIA and other organizations as well, assumptions must not be made that the cells are similar to previous cell lines developed from the same organ in the same species. The SEM studies of the in-vitro cell lines with increasing population doublings represent some of the first data gathered with this particular instrument (SEM) on aging cells in culture. The results show that surface changes do indeed occur on aging cultured cells and suggest further effort be spent on the membrane dynamics of aging cells. The TEM comparison of cytoplasmic organelles show that there is a general structural difference in dense bodies between in-vitro and in-vivo preparations. The ringed dense bodies seen in cultured cells are also

found in diseased cells of animals and man in-vivo. This suggests a careful analysis of the environment in which cultured cells are grown and perhaps more effort be expended on the development of better media in which to grow them.

Proposed Course: The TEM and SEM studies of normal aging animals tissues will be continued. The SEM examination of the aging digestive system is scheduled for completion by this year (1978). The dietary restricted mice have been examined at 12 months of age and the 24 month restricted groups are scheduled to be sacrificed in August of 1978. Squirrel monkey material will be examined for comparison of primate and rodent cellular aging at the EM level. A further examination of dense bodies will be made using in-vivo and in-vitro cells and histochemical techniques such as acid phosphatase.

Publications:

Miquel, J. and Johnson, J. E., Jr.: Senescent changes in the ribosomes of animal cells in vivo and in vitro. Mechanisms of Ageing and Development, in press.



Annual Report of the Clinical Physiology Branch, NIA  
October 1, 1977 through September 30, 1978

SUMMARY

The major addition to the research program was the initiation of the Longitudinal Study of Aging in Women. It started essentially on the twentieth anniversary of the initiation of the male study. This was accomplished despite minimal increase in staffing and only by dint of fine cooperative efforts of the scientists and technical and administrative staff members. It will, unfortunately, be necessary for us to take more than one 2-year cycle of examinations to build up to a study population size equal to that of the men (650) and we may have to undertake some scheduling stretch-out for the men as well unless staff increases can be accomplished.

In the first six months of the female study, we studied 85 women. Changes in formatting of the various test instruments were accomplished by a new staff member (a nurse-epidemiologist), and the services of a nurse-practitioner who specializes in pelvic and breast examinations were obtained through the Baltimore City Hospitals. Other important scientific additions to the study include the initiation of a comprehensive survey of immunological factors, expansion of the non-invasive assessment of cardiovascular variables, and a survey of oral and dental variables.

The current status of the male study shows 1104 subjects with 6406 total visits. One hundred seventy-three (173) have died; 287 have withdrawn from the study (including those who have become ill and those who have moved away), leaving 644 active participants.

Dietary variables have been assessed in the subjects of the BLSA from 7-day dietary diary records. These estimates are of high quality in this study because of several factors: (1) the subjects are of very high educational level, (2) trained dietitians taught them how to describe meals both qualitatively and quantitatively, (3) the 2-1/2 day stay on each visit enabled the dietitians to combine didactic instruction with practical instruction at mealtimes, and (4) subjects had high motivation to provide accurate data. A detailed analysis is being conducted on subjects who provided data in each of three 5-year epochs from 1961 to 1975. The subjects were mainly 35-79 years of age. Both age changes and secular changes in dietary factors have been examined. Total caloric intake decreased both with age and secularly over the 15-year period. As an example, 50-54 year old subjects in 1961-65 consumed more calories than did 50-54 year old subjects in 1971-75; furthermore, the cohort that was 50-54 years of age in 1961-65 and that was therefore 60-64 years of age in 1971-75 showed a decrease in caloric intake as they aged. These changes were generally true for each of the nine age quintiles.

The secular decrease in total calories occurred because of a decrease in fat, protein, and carbohydrate (CHO). Furthermore, saturated fatty acid (S) and cholesterol intake decreased. In contrast, there was a secular increase in polyunsaturated fatty acid (P) intake, so that the P/S ratio increased by 26% (0.34 to 0.43) over the span of time studied.

The decrease with age in total calories was clearly evident throughout the 15 years of the study. This decrease is largely due to a lower intake of fat with increasing fat. The percent of total calories derived from protein is remarkably constant at all ages, while that derived from CHO actually increases with age. This latter finding is of especial interest since it shows that the decrease in glucose tolerance with advancing age cannot be attributed to a shift to a relatively lower CHO intake.

It is also of importance that the secular shift in P/S ratio occurred in our subjects in association with a secular fall in serum cholesterol levels. This association thus far has been examined only for groups of subjects. We will in the coming year analyze the dietary and serum variables on a subject by subject basis to test whether there is, in fact, an association between P/S and serum cholesterol changes.

In a cooperative study with a graduate student from the University of Michigan, a first effort was made to relate physiological variables to a diverse group of variables which, for convenience, are called "life style variables" (these include education, marital status, caloric intake, smoking, and mortality status). For this analysis, data obtained on the first visit was analyzed on 1086 men. Performance levels were corrected for age by judging each individual's performance against those of his age cohorts. While this should be considered only as a first effort at intercorrelating a large number of diverse variables, the results are of interest. Those biological variables which were most strongly related to the life style variables involved performance of physical tasks. While those biological variables which require either very little or no physical performance by the subjects showed the weakest correlations.

Work in the Endocrinology Section continues to identify age-related changes of hormone secretion and action and to elucidate the mechanisms involved in these events. In terms of the initial phases of hormone action, the Section has previously described alterations of hormone receptor concentrations in several tissues. We are now continuing our efforts to quantitate such changes for cell surface  $\beta$  adrenergic receptors in order to relate receptor number and affinity to known age-related alterations of adenylate cyclase (liver) and lipolysis (fat). Quantitation efforts in liver have so far been unrewarding, but the techniques appear satisfactory for fat. Intracellular receptors for glucocorticoid hormones also are receiving attention, since these have been shown to decrease with age in brain and fat cells. In the latter, decreased receptor number correlates with decreased effectiveness of the metabolic

action of glucocorticoid. This work now involves the development of techniques for study of receptor synthesis rates. Early findings indicate that decreased synthesis accounts for the previously demonstrated age-related changes of glucocorticoid receptor concentration. This change is relatively selective, since overall protein synthetic rate is not affected during aging of the cell type used (fat). Another type of cell surface receptor, that for the protein hormone, gonadotropin, has been shown to decrease in number during aging of the testis. In this case, the functional significance of the change has also been assessed. Despite a decrease of about 30% in receptor number, cAMP generation is unaffected, suggesting that adenylate cyclase is adequately stimulated despite decreased receptors. Since a defect in gonadal steroid production has also been identified, a biochemical "lesion" has been located between the formation of cAMP and some step in or prior to steroid synthesis.

Increasing evidence is being accumulated from the past year's results that membrane changes may be important in age-related alterations of hormone action. For example, the ability of the senescent rat adipocyte membrane to transport glucose appears to lose its facility to be regulated by a variety of hormones and chemical agents. Other probes of adipocyte membrane integrity indicate age-related changes in response to perturbations by guanine nucleotides and ions. In addition, the stability of the liver's cell membrane decreases, a change which is associated with altered adenylate cyclase (and ATPase) activity.

At the level of the intact human organism, studies of reproductive physiology in the GRC's study population has given some unexpected results. Data for cross sectional studies have reported an age-related decrease of testosterone in plasma. Our subjects do not exhibit this change, nor do we see the alterations of dihydrotestosterone or estrogens reported by others. We do observe, as have others, alterations of pituitary gonadotropins. Our immediate conclusion is that factors such as alcohol intake, physical activity, obesity, etc., may be critical and that the use by others of less well-defined populations may have obscured the real state of affairs. Other possibilities (e.g., age-related alterations of diurnal rhythms) will be explored to attempt to account for our differences. Another preliminary but intriguing observation is one of total lack of correlation between level of sexual activity and plasma hormone levels.

The basic biochemistry of adenylate cyclase has been studied in a series of experiments which show the existence in liver of proteins which act as "cofactors" for adenylate cyclase. These materials are currently being isolated. Other biochemical work has helped elucidate the character of renin substrate and has provided a new technique for the isolation of renin and its separation from closely related enzymes.



Another significant addition to the BLSA is being accomplished via a contract with the Cardiovascular Division of the Johns Hopkins University. Unidimensional echocardiography at the GRC has shown some interesting age differences in both the structure and the function of the heart and these results have been reported. With advancing technology, much more information can now be obtained with the bi-dimensional ECHO. Although we did not have adequate staff or funds to set up the newer procedure, fortunately we were able to arrange for our participants to be studied at Hopkins. Only 80 have been studied to date; data analysis will await the completion of a larger number of tests.

At the same time, <sup>201</sup>Thallium myocardial imaging has been added to our non-invasive assessment of age changes in cardiovascular function. This measure of coronary blood flow will be compared to assessments by the stress ECG technique and by the ECHO. Again, these studies were just initiated and analyses await accumulation of a larger experience.

Previous work from the Cardiovascular Section has demonstrated that myocardial muscle from aged animals has diminished inotropic and chronotropic responses to catecholamines in rat, dog, and man. We have now been able to focus down on the site of this age decrement in rats by studying responses of the interventricular septum to isoproterenol (IP) stimulation. Both the control levels and the IP-stimulated levels of cAMP, of protein kinase and of the protein-kinase activation ratio show no age differences. Furthermore, there is no age difference in the mechanical response to dibutyryl cAMP. Thus, the age defect in mechanical response to catecholamines lie distal both to the receptor and to the protein kinase activation step.

A major addition to the BLSA occurred in July, 1977, with the initiation of a comprehensive survey of variables in the immune system. There has already been substantial progress from the testing of the first 200 participants. Some of these subjects will be restudied in the coming year to test for relatively short-term replicability of the tests and to initiate the study of true longitudinal age changes. T cell proliferative response to mitogens decreased with age beginning at the 5th decade and was markedly reduced at the 6th decade. T cell cytotoxic activity was also lowered beginning in the 6th decade. Total lymphocyte count begins to decrease early in adult life among the Longitudinal subjects, but the study of the differentiation of these lymphocytes into specific cell types has only just begun.

Immune lymphocyte function has been assessed by cell proliferation (thymidine incorporation) and quantitating immunoglobulin forming cells in response to mitogens. The amount of pokeweed mitogen needed for inducing immunoglobulin synthesis was much lower than the optimal amount required for lymphocyte proliferation. There was no apparent correlation between these two parameters. The development of these techniques will enable us to analyze lymphocyte function as it is influenced by age.



Studies have also been initiated on nucleic acid synthetic pathways in lymphocytes by using the inhibitor chloramphenicol. Many of the characteristics of this system have been worked out and it was found that lymphocytes from older individuals are much more susceptible to the suppressive effects of the drugs than are cells from our younger subjects.

Among the variety of studies conducted in this human population, two did not show any age differences: serum immunoglobulin levels and antibody-forming ability of the peripheral blood lymphocytes.

This battery of results from the first year of this study is promising since very significant age differences have been revealed. The relation of these changes to the clinical history of these subjects will be of great importance to follow as they age.

Studies conducted using the murine system as an experimental model have shown that T cell cytotoxic activity as assessed in vitro decreases as a function of increasing age. This correlates well with the similar studies done using the Longitudinal subjects. In addition, the murine studies have indicated that a lymphoid suppressor cell is responsible for the decreased T cell cytotoxicity.

Other studies conducted using the murine system have centered around the antibody response to a T-cell independent antigen. Because it is well established that T-cell function decreases with age, this antigen would be useful to assess a B-cell function in aging mice irrespective of the T-cell compartment. Such studies have shown that old mice respond to a lesser extent than young control mice. The lower response is related to a reduced proliferation of the lymphoid cells derived from the older mice. The cellular mechanism that could explain the reduced response in the aging mice is being investigated.

Studies involving the feasibility of storing murine cells at low temperatures have shown that cells frozen under controlled conditions are able to retain the majority of their reactivity to several mitogens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00001-08 CPB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less)  Metabolic Studies of Aging in Man																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">R. J. Hershcopf</td> <td style="width: 30%;">Clinical Associate</td> <td style="width: 20%;">CPB NIA</td> </tr> <tr> <td></td> <td>E. C. Hadley</td> <td>Staff Fellow</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>S. P. Tzankoff</td> <td>Staff Fellow</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>R. Andres</td> <td>Chief, CPB</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>J. D. Tobin</td> <td>Medical Officer</td> <td>CPB NIA</td> </tr> </table>			PI:	R. J. Hershcopf	Clinical Associate	CPB NIA		E. C. Hadley	Staff Fellow	CPB NIA		S. P. Tzankoff	Staff Fellow	CPB NIA		R. Andres	Chief, CPB	CPB NIA		J. D. Tobin	Medical Officer	CPB NIA
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SECTION Metabolism Section																						
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<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
<p>           A test of the predictive power of four physiological variables which change with <u>age</u> showed that poor performance on <u>creatinine clearance</u>, <u>forced expiratory volume</u>, and <u>systolic blood pressure</u> was associated with <u>mortality</u>. Poor <u>glucose tolerance</u>, however, did not predict mortality and, therefore, may not be of great significance. <u>Glycosylated hemoglobin</u> levels may be a better test of the hypothesis that relative <u>hyperglycemia</u> is harmful even in those not overtly diabetic; several new sources of variance for this variable have been found and this should add to the reproducibility and reliability of the assay. Basal levels of <u>growth hormone</u> and of <u>glucagon</u> do not change with age in our subjects and, therefore, do not contribute to decline in glucose tolerance with age.         </p>																						

GRC/CPB-22

### Project Description:

Objectives: This project is primarily concerned with furthering our understanding of the relations between physiological aging processes and specific diseases in the elderly. The major focus is on glucose homeostasis and diabetes mellitus, and on aging and secular changes in serum lipids. Studies are directed at long-term follow-up of volunteers in the Baltimore Longitudinal Study of Aging in order to acquire actuarial data for judging the significance of varying levels of performance on the diagnostic tests for diabetes mellitus which are in clinical use. Other studies are directed at discovering the pathophysiologic mechanisms underlying the age changes in performance on these tests.

Methods Employed: Standardized tests (oral and IV glucose tolerance, cortisone-primed oral glucose tolerance, and IV tolbutamide response test) are given on a rotating basis, one test on each visit unless a special glucose clamp study is performed. Glucose is measured by an automated glucose oxidase procedure. Insulin, glucagon, and growth hormone are measured by radio-immunoassay. Hemoglobin glycosylation is determined by ion-exchange chromatography for hemoglobin A<sub>1</sub>, and a direct assay of glycosylated lysine residues in hemoglobin hydrolysates is being explored. Subjects are the male and female volunteers of the Baltimore Longitudinal Study of Aging.

Major Findings: (1) An analysis of four physiological variables in the Longitudinal Study has been carried out as the first of a series of studies on the predictive power of diverse levels of performance as risk factors for mortality from all causes. The variables were selected because each showed decided changes with age and each is associated with a distinct disease state. The functions tested were: (a) renal (24 hour creatinine clearance), (b) pulmonary (forced expiratory volume in one second), (c) cardiovascular (systolic blood pressure), and (d) glucose metabolism (oral glucose tolerance). Normative age standards were computed from a subset of the population which was "clinically clean" with respect to each variable. For each individual, a standard T-score was computed for each of the 4 tests. At the time of these analyses, 162 participants had died; T-scores on this group were compared to the scores for those who remained alive. For three of the variables, creatinine clearance, FEV, and S-BP, the group which subsequently died had significantly lower scores than those who survived. This suggests that declines in "performance" in those variables should not be considered as physiological or normative, since they were associated with mortality. For glucose tolerance, however, the scores for the dead and alive groups did not differ; this preliminary examination suggests that "deterioration" of performance in this system (short of overt diabetes) may simply represent normal non-harmful aging.

(2) Assessment of glycosylated hemoglobin levels in the Longitudinal subjects should provide a better estimate of the time-integrated blood glucose concentration over a period of the several months prior to study. Thus,



theoretically, it should provide a more valid test of the hypothesis that those subjects with relative hyperglycemia will have a poorer outcome with respect to macro- and micro-angiopathy and to longevity. While too few subjects have been tested to date even to permit correlation between the glycosylated hemoglobin level and glucose tolerance, some previously undescribed sources of interassay variability have been discovered:

(a) There is a rapid conversion of Hgb A1 from the oxy- to the cyanmet-form in column eluates. This can result in considerable variation if spectrophotometric determinations are made at the usual wavelength of 415 nm. (b) There is great temperature sensitivity of the assay, so that a difference of 2 degrees C can result in a 10% difference in the determination. To deal with this interassay variability, we convert hemolysates to the cyanmet derivative and store them in liquid nitrogen.

(3) Basal levels of glucose, insulin, glucagon, and growth hormone were measured in 187 subjects from 25 to 93 years of age. Obesity was computed by anthropometry (method of Behnke). None of the hormones varied with age, but obesity did increase with age in these subjects. Insulin and glucose levels did correlate positively with obesity, and these correlations were independent of age. Thus, decline in glucose tolerance with age cannot be attributed to increases in either glucagon or growth hormone levels.

(4) The end of our 9th two-year cycle of tests on June 30, 1977, has been selected as the end-point for carrying out extensive simple and multivariate analyses of variables in the study. A major effort was therefore made this past year to verify the extensive stored data in the glucose area. This chore has been completed so that longitudinal analyses of these tests, relations among the tests, and relative predictive values of the tests can be computed in the coming year.

#### Significance to Biomedical Research and the Program of the Institute:

The remarkable prevalence (50%) of abnormal glucose tolerance in the older population of the United States coupled with the increased morbidity and mortality of patients with true diabetes mellitus demands a delineation of the effects of aging on the pathophysiology of carbohydrate homeostasis. The tests of "abnormality" in performance levels at different ages will be certain end-points which will develop with the passage of time: development of overt diabetes, retinopathy, coronary heart disease, and peripheral atherosclerosis; decline in creatinine clearance; mortality rate; rates of "physiological aging" in other organ systems.

Proposed Course: Further analyses of metabolic data collected from January 1, 1963, to June 30, 1977, including multivariate computations among the diagnostic tests for diabetes and related variables (age, obesity, serum lipids, BP, cigarette smoking, etc.) and specific end-points related to diabetes (macroangiopathy, such as coronary, cerebrovascular, and peripheral atherosclerosis, and microangiopathy, such as retinal, neurological including sexual potency and renal changes).



Publications:

Andres, R.: Influence of obesity on longevity in the aged. In: Proceedings of the Conference on Aging: A Challenge to Science and Social Policy, held 4/29/77, Institute de la Vie, Vichy, France. Pergamon Press, Ltd, Oxford, Great Britain. In Press.

Tobin, J.D.: Physiological indices of aging. In: Proceedings of the Conference on Aging: A Challenge to Science and Social Policy, held 4/29/77, Institute de la Vie, Vichy, France. Pergamon Press, Ltd, Oxford, Great Britain. In Press.

Helderman, J.H., Vestal, R.E., Rowe, J.W., Tobin, J.D., Andres, R.: The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man: The impact of aging. J. Gerontol. 33: 39-47, 1978.

DeFronzo, R.A., Tobin, J.D., Rowe, J.W., and Andres, R.: Glucose Intolerance in Uremia: Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. J. Clin. Invest. In Press.

DeFronzo, R.A., Cooke, C.R., Wright, J.R., and Humphrey, R.L.: Renal function in patients with multiple myeloma. Medicine 57: 151-166, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00002-16 CPB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  The Gastrointestinal Hormone Mediation of Insulin Release		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	D. Elahi J. D. Tobin R. Andres	Staff Fellow Medical Officer Chief, CPB
		CPB NIA CPB NIA CPB NIA
COOPERATING UNITS (if any)		
	R. T. Moxley, III, Dept of Medicine, Johns Hopkins Univ. D. H. Lockwood,                      "   "   "   "   "   " J. M. Amatruda,                      "   "   "   "   "   " T. Pozefsky,                          "   "   "   "   "   "	
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 2.6	PROFESSIONAL: 1.3	OTHER: 1.3
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>           This study is designed to investigate the role of the <u>gastro-intestinal tract</u>            as an endocrine gland in the maintenance of <u>glucose homeostasis</u>. Among the            identified <u>gut hormones</u>, <u>gastric inhibitory polypeptide</u> (GIP) appears to be            the major <u>insulin secretagogue</u>. The glucose clamp technique has demonstrated            the role of the endogenously-released hormone under in vivo conditions in            normal man. GIP infusion studies have shown that the hormone has the same            effects when exogenously administered. Furthermore, <u>jejunoileal bypass</u> for            morbid <u>obesity</u> results in marked reduction in insulin response to an oral  <u>amino acid</u> load.         </p>		

## Project Description:

Objectives: The studies are designed to examine the role of the gastrointestinal tract on insulin responses to ingestion of certain nutrients in man. Responses to the endogenously-released and exogenously-infused hormone are compared. The perfused rat pancreas is used as a model for studies of age changes in insulin secretion.

Methods Employed: Subjects are normal volunteers and morbidly obese patients undergoing jejunoileal bypass surgery. The glucose clamp technique provides control of the blood glucose concentration. Glucose and amino acids are measured by automated glucose-oxidase and chromatographic techniques. Insulin and GIP are measured by radio-immunoassay. Single pass rat pancreas perfusion via the Grodsky method is used.

Major Findings: (1) The primary set of physiologic studies on the role of endogenously released GIP in man have been published by Andersen et al. They show: (a) the close similarity in time course for the rise in plasma GIP and insulin levels; (b) the graded response of the beta cell to GIP to the ambient blood glucose concentration perfusing the pancreas (from zero response at euglycemia to remarkably high responses when hyperglycemia is marked); (c) the threshold of glycemia required for GIP to act as an insulin secretagogue is 20-30 mg/dl above the normal basal glucose concentration.

(2) Further studies on the enteric insulinotropic hormone were done in collaboration with scientists at the Johns Hopkins University. Oral and IV amino acid tolerance tests were performed before and after jejunoileal bypass surgery for morbid obesity on 31 patients. Preoperatively, a 30 g mixture of amino acids given orally evoked a larger peak insulin response than did an IV load which yielded comparable plasma amino acid elevations; this difference is presumably secondary to the effect of an enteric insulinotropic factor. Four months postoperatively, the basal insulin concentrations had fallen and the response to IV amino acids was preserved when response was expressed as the percentage increase over basal. In contrast, the insulin response to an oral amino acid load was significantly blunted.

Although gastric inhibitory polypeptide (GIP), the probable major insulinotropic gut hormone, was not measured in these studies, the differential response of IV and oral loading after bypass indicates that the source of an enteric factor(s) responsive to oral amino acids was removed by the surgery.

(3) The rat pancreas perfusion technique has been established and validated in our laboratory. Characteristic biphasic responses to hyperglycemia occur and a graded dose:response (glucose:insulin) curve is present. As is customary, these techniques were established using retired male breeders; studies with young and old rats from the GRC colony have just begun.

(4) The manuscript of the GIP infusion studies in man has been submitted for publication.

Significance to Biomedical Research and the Program of the Institute: The high prevalence of altered glucose tolerance in aging and obesity, as well as the high incidence of adult-onset diabetes mellitus, require further understanding of factors which contribute to this process. In addition, an understanding of similarities and differences in the pathophysiology of the various categories of glucose intolerance provides the hope for more and improved methods of treatment. Pathological states associated with alterations in gastro-intestinal hormones are only superficially understood currently, and further investigation of these hormone systems adds greatly to a new area of medical knowledge.

Proposed Course: Manuscripts will be prepared on several completed aspects of this work: (1) the effects of age, obesity, and diabetes in man, (2) the effects of fat ingestion on GIP secretion and effects in man, (3) interactions of arginine infusion, glucose infusion, and endogenous GIP after glucose ingestion in man. The perfused rat pancreas model will explore the effects of age in a strain which does not become overtly diabetic with increasing age.

Publications:

Andersen, D.K., Elahi, D., Brown, J.C., Tobin, J.D., and Andres, R.: Oral glucose augmentation of insulin secretion: Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. J. Clin. Invest 62: 152-161, 1978.

Moxley, R.T., III, Lockwood, D.H., Amatruda, J.M., Tobin, J.D., and Pozefsky, T.: Loss of insulin response to ingested amino acids after jejunoileal bypass surgery for morbid obesity. Diabetes 27: 78-84, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00009-04 CPB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Age Changes in the Mechanical Properties of the Cardiovascular System														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">H. A. Spurgeon</td> <td style="width: 40%;">Physiologist</td> <td style="width: 10%;">CPB, NIA</td> </tr> <tr> <td></td> <td>E. G. Lakatta</td> <td>Chief, Cardiovascular Section</td> <td>CPB, NIA</td> </tr> <tr> <td>Other:</td> <td>P. T. Thorne</td> <td>Head, Technical Development</td> <td>OSD, NIA</td> </tr> </table>			PI:	H. A. Spurgeon	Physiologist	CPB, NIA		E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA	Other:	P. T. Thorne	Head, Technical Development	OSD, NIA
PI:	H. A. Spurgeon	Physiologist	CPB, NIA											
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA											
Other:	P. T. Thorne	Head, Technical Development	OSD, NIA											
COOPERATING UNITS (if any) F. C. P. Yin, Div. of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions														
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch SECTION Cardiovascular Section INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANHOURS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">1.2</td> <td style="text-align: center;">1.1</td> <td style="text-align: center;">.1</td> </tr> </table>			TOTAL MANHOURS:	PROFESSIONAL:	OTHER:	1.2	1.1	.1						
TOTAL MANHOURS:	PROFESSIONAL:	OTHER:												
1.2	1.1	.1												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINDS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) Mechanical properties of heart muscle determine to a great extent the way in which biochemical events in the cell are transduced into muscle tension and ultimately into development of pressure in the heart. Changes in the <u>mechanical properties of the aged heart</u> could account for the observed changes in cardiac performance as determined by a variety of indices. This program uses <u>vibration techniques</u> to measure the components of a <u>cardiac</u> and vascular muscle which determine the overall " <u>stiffness</u> ," and measure the <u>age induced effect of the stiffness</u> . Additional studies are purposed to determine the relationship of stiffness to the <u>sarcomere</u> rather than to the gross muscle, and to determine whether the extent to which the <u>myofilaments</u> are <u>activated</u> influences their <u>stiffness properties</u> .														
GRC/CPB-29														

## Project Description

Objectives: The initial studies of this laboratory have demonstrated that dynamic stiffness is increased in senescent rat myocardium, compared to the young adult (Spurgeon et al., Am. J. Physiol. 232: H373-H380, 1977). The current objective is to determine whether the change in stiffness relates to the moderate increase in heart mass that occurs with age. In addition, projects have been undertaken to (1) attempt to relate muscle stiffness to sarcomere stiffness and (2) determine whether dynamic stiffness measured during contraction relates to the level of activation of the myofilaments.

Methods: (1) Isolated superfused trabeculae carneae from adult and senescent rats are clamped in an apparatus which constrains the muscles to an isometric length. Using techniques developed from vibration analysis, the stiffness of the sample of cardiac muscle is determined as a stress/strain ratio by subjecting the isometric muscle to sinusoidal length perturbations, typically of 0.022 mm, at frequencies from .001 to 100 Hz. The muscle is caused to contract isometrically during the study, enabling computation of the changes in dynamic stiffness associated with the contractile process. The resulting tension, which varies in step with the sinusoidal length changes imposed, is the strain produced stress, and by definition represents the total stiffness of the muscle. These studies have been performed in muscles of adult and senescent control rats and in muscles taken from adult rats after 16 weeks of mild aortic banding. This procedure results in a 15% increase in left ventricular mass and in some ways mimics the hypertrophy seen in the senescent rat heart.

(2) An apparatus is currently being constructed that allows monitoring of sarcomere length during contraction. This procedure utilizes diffraction of a laser light signal through the muscle and a series of optical lenses onto photomultiplier tubes. The change in mean sarcomere length is related to the distance between the zero and first order bands on the diffraction pattern and this can be quantitated. When completed, the procedure in (1) will be performed, utilizing sarcomere length strain rather than changes in gross muscle length to measure stiffness.

(3) Changes in activation resulting from changes in bathing solution [ $\text{Ca}^{++}$ ], altered patterns of stimulation, or pharmacologic agents, are employed and stiffness is measured as in (1). It is proposed that these studies will be performed as in (2) when this apparatus is complete.

Major Findings: An initial group of experiments on the influence of the level of activation on dynamic stiffness has been completed and the results indicate that stiffness during contraction does in

canine trabecular muscles, vary directly with the level of activation. However, this does not appear to be the case in the rat muscle and additional studies will be made.

Significance to Biomedical Research and the Program of the Institute: The implications of the completed study have rather wide impact. First, the indication of increased stiffness in both passive and active states of the aged heart muscle suggests the age related change of this important measure occurs not only in the passive supportive structure of the muscle, but implies changes in the active contractile elements as well. Second, the suggestion that the old heart stiffness versus tension curve exhibits a steeper slope, and that this slope increases with age in both the passive and active state indicates a fundamental change in the muscle with age. Third, although it is widely held that the old heart is incapable of extended performance under stress, the efficiency of the aged heart may actually be increased. Assuming a series model, tension developed is the product of distance the contractile element shortens and the stiffness of the element. For tension to remain relatively unchanged across age, the amount of shortening required may decrease by as much as 25%. Although tension development fails to decrease markedly with age, the tension developing mechanism might well decrease, and the magnitude of that decrease may prove greater than suspected because of the "masking" effect of increased stiffness. In addition to defining age changes in cardiac muscle, studies relating stiffness during contraction to activation and sarcomere length may significantly contribute to our current understanding of muscle physiology per se.

Proposed Course: (1) To continue studies of dynamic stiffness in muscles from hypertrophied animals. (2) To continue studies investigating the influence of the level of activation on dynamic stiffness. In order to avoid potential artifacts, these studies must be validated utilizing methods as described in (2).

Publications:

Lakatta, E. G.: Excitation-Contraction Coupling. In Weisfeldt, M. L. (Ed.): The Aging Heart. New York, Raven Press (in press).



## PERIOD COVERED

October 1, 1977 to September 30, 1978

## TITLE OF PROJECT (80 characters or less)

Echocardiographic Assessment of the Left Ventricle and Mitral Valve in Aging Man

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. L. Fleg Staff Cardiologist CPB, NIA  
E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Other: None

## COOPERATING UNITS (if any)

F. C. P. Yin, Div. of Cardiology, Dept. of Medicine, Johns Hopkins Medical Institutions

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SECTION

Cardiovascular Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MAN-YEARS:

0.9

## PROFESSIONAL:

0.8

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Earlier echocardiographic work from this laboratory has shown that the left ventricular thickness and aortic root diameter increase as a function of age whereas cavity size, ejection fraction and velocity of shortening are unchanged and mitral valve closure slope is reduced. Since this initial project was limited to men with a maximum blood pressure of 140/90, we have extended this study to women and extended the blood pressure range for entrance into the study of both sexes across a spectrum of ages (18-95).

A research contract involving the cardiology division of Johns Hopkins Hospital to evaluate cardiac anatomy and function at rest and during exercise began on 1 November 1977 and will extend for three years. Two-dimensional exercise echocardiography and thallium<sup>201</sup> myocardial perfusion scanning will be employed. This is discussed in the appendix to this report.



Project Description:

Objectives: (1) Echocardiography is an ideal non-invasive method for assessing age-related changes in cardiac anatomy and function. We hope to determine the pattern of these changes in normal women and compare the results to those of normal men of the same ages. Hypertension is known to induce thickening (hypertrophy) of the left ventricular walls. Echocardiography will be utilized to determine the extent of this hypertrophy, and whether increases in blood pressure that occur with age are correlated with thickening of the left ventricle.

(2) Non-invasive techniques are employed in man to measure the function of the left ventricle while it is subjected to a known amount of stress. The rationale is that diseased or failing hearts may appear to function normally when not subjected to undue stress, but reveal abnormalities when subjected to even a moderate degree of stress. Previous animal experiments from this laboratory have demonstrated that the aged but otherwise healthy stressed heart has certain functional impairments. Comparable information in man is lacking. Utilizing modern techniques, it is possible to examine this question in man. By comparing the performance of normal (by standard criteria) young and aged hearts both unstressed and during the imposition of a controlled degree of stress, one is able to assess the degree of impairment in heart function due to the aging process.

Methods: Echocardiograph assessment of the aging heart includes two major projects. (1) Normal men and women in the Baltimore Longitudinal Study and those with uncomplicated hypertension receive resting echocardiograms. Left ventricular wall thickness, systolic and diastolic dimensions and rate of endocardial shortening, mitral valve closure rate, plus aortic root and left atrial dimensions are measured.

(2) The second study utilizes the echocardiogram to measure left ventricular function during imposition of a stress. A predetermined increase in peripheral blood pressure is induced sequentially by isometric handgrip exercise or by infusion of an epinephrine-like drug (phenylephrine). Measurements are made before and after temporary blockade of the sympathetic nervous system achieved by infusing the beta-adrenergic blocking drug propranolol. Electrocardiograms and blood pressure are recorded simultaneously with the echocardiogram. Left ventricular function is assessed by measuring changes in the diastolic and systolic dimensions and velocity of shortening of the endocardium during the various interventions.

Major Findings: (1) The results in normal men were presented by Gerstenblith et al., Circulation 56: 272-278, 1977. The data on

women and hypertensives are currently being gathered and analyzed.

(2) The results of Project II demonstrate that when beta-adrenergic tone is eliminated, the left ventricular cavity dilates in response to phenylephrine more in the aged than in the adult population.

Significance to Biomedical Research and the Program of the Institute: Information concerning the anatomic and functional aging changes of the normal human heart is critical to understanding the aging process of the cardiovascular system. Similar information is needed in aged "hypertensives" (over 10% of the population) to examine the effects of hypertension on the heart and their interrelationships with the aging process.

Proposed Course: The current project will be continued at least through the next fiscal year. The feasibility of an echocardiographic study examining the effects of bed rest on cardiac function will be considered.

Publications:

Yin, F. C. P., Raizes, G. S., Guarnieri, T., Spurgeon, H. A., Lakatta, E. G., Fortuin, N. J., and Weisfeldt, M. L.: Age-associated decrease in ventricular response to haemodynamic stress during beta-adrenergic blockade. Brit. Heart J. (in press).

Appendix

Contract Number: N01-AG-7-2129

Contract Title: Non-invasive Assessment of Cardiac Structure and Function in Aging Men and Women

Contractor: Johns Hopkins University, Baltimore, Maryland

Investigators: G. Gerstenblith, Asst. Prof. of Medicine

M. L. Weisfeldt, Dir., Div. of Cardiology

J. Weiss, Asst. Prof. of Medicine

L. Becker, Asst. Prof. of Medicine

Money Allocated: \$237,909.00

Objectives: Two-dimensional echocardiography is a new technical development which allows determination of cardiac anatomy and function in much the same manner as routine echocardiography, with the important addition that an entire plane of the heart can be visualized at once rather than a simple "ice pick" view, allowing greater accuracy in the determination of heart chamber shape, size and function. The goal is to examine 750 subjects from the Baltimore Longitudinal Study over a three year period (ages 18-95) during rest and maximal semi-supine bicycle exercise to determine age related differences in regional and global myocardial function.

Thallium<sup>201</sup> myocardium imaging allows non-invasive assessment of regional left ventricular blood flow and will be used to determine the incidence, severity and prognostic implications of ischemic heart disease in Baltimore Longitudinal Study participants. The predictive values of this technique will be compared to that of stress electrocardiography and two-dimensional echocardiography. About 150 new subjects will be studied for each of three years. In year 5 the subjects in year 1 will be retested.

Methods: Subjects will perform maximal graded semi-supine bicycle ergometry and simultaneous ECG and blood pressure monitoring every minute. Two-dimensional echograms will be made in longitudinal and cross-sectional views of the left ventricle at rest, maximal exercise and during recovery with regard to the following echocardiographic indices (1) myocardial mass, (2) left ventricular chamber size, (3) mean and maximal velocity of fiber shortening and lengthening, (4) percent change plus mean and maximal velocities of regional left ventricular systolic thickening and diastolic thinning, (5) percent, mean and maximal rates of change in regional left ventricular radius and (6) duration of left ventricular ejection and filling.

Thallium imaging will be performed in four views following peak exercise of a multi-stage maximal treadmill test. Subjects found to have perfusion defects after stress are re-imaged in 2 hours (without further nuclide injection) to determine whether there is any "filling in" of the defect.

Major Findings: To date, approximately 80 subjects have undergone each of the two procedures. Data analysis will be performed after one full year of the study.

Significance to Biomedical Research and the Program of the Institute: Two-dimensional echocardiography will allow detailed non-invasive analysis of regional and global cardiac wall motion in normal adults representing eight decades and in subjects with coronary artery disease. Such information should help to define

early pathological changes in cardiac muscle function.

Thallium<sup>201</sup> myocardial imaging will permit the non-invasive detection of coronary disease in a normal population. Periodic repeat examinations will allow the calculation of incidence of new disease as well as progression rates in subjects with manifest disease. Such information was previously available in coronary arteriography, an invasive, costly and more dangerous procedure.

Proposed Course: Both two-dimensional echocardiography and thallium<sup>201</sup> myocardial scanning should prove valuable tools for longitudinal assessment of cardiac wall motion and myocardial perfusion respectively and should allow the response to numerous medications and physiological interventions to be determined, both in normals of various ages and in patients with heart disease.



## PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Response to Cardiac Glycosides in Senescent Dogs, Rats, and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. Guarnieri Clinical Associate (DOD 6/30/78) CPB, NIA  
E. G. Lakatta Chief, Cardiovascular Section CPB, NIA  
H. A. Spurgeon Physiologist CPB, NIA

Other: J. P. Froehlich Medical Officer LMA, NIA

## COOPERATING UNITS (if any)

G. Gerstenblith, Div. of Cardiology, Dept. of Medicine, Johns  
Hopkins Medical Institutions

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SECTION

Cardiovascular Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MAN-YEARS:

.85

## PROFESSIONAL:

.80

## OTHER:

.05

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of the role of aging on the: (1) direct effect of cardiac glycosides in isolated cardiac muscle from rats; (2) effect of cardiac glycosides on electrophysiology and mechanical performance in intact dogs; (3) effect of cardiac glycosides and  $\text{Na}^+\text{K}^+$  ATPase inhibition in cardiac muscle from both rodent and canine species; and (4) effect of age on ouabain infusion in normal man.

GRC/CPB-37

**Project Description:**

Objectives: The objectives of this study are to characterize the effect of age response to cardiac glycosides both in the intact organism and in isolated heart muscle.

Methods: (1) Intact dogs.

(a) Unanesthetized awake dogs are given rapid bolus injection of acetylstrophanthidin. This is a rapid acting glycoside. Doses are repeated in 5  $\mu\text{g/kg}$  increments each 30 minutes until the end point of the study - ventricular tachycardia ensues. The dose required to produce this toxic effect is compared in both young adult and senescent beagles. Serum levels of the drug are monitored prior to each dose.

(b) Anesthetized dogs are instrumented with a catheter tipped micro-monometer placed in the left ventricle and a pacing catheter inserted into the right ventricle. The dose response curve obtained in a given dog in the awake state is repeated in the anesthetized state. The inotropic response of pressure and rate of pressure development are measured at a given paced rate; in addition toxic effects on the ECG are simultaneously measured.

(c) Beta-blockade. On a third day (nonsuccessive), part (b) is repeated in the presence of propranolol, a beta-adrenergic blocking drug. Thus in a given dog, electrical responses are measured in the awake state, and both electrical and mechanical responses are measured in the anesthetized state. When the dog is subsequently sacrificed at a later date, muscle is removed from the heart and the direct effect of the glycoside on the muscle is measured (see below).

(2) Isolated muscle. Trabecular muscles are isolated from hearts of the rat and dog and the responses to ouabain (rat) and acetylstrophanthidin (dog) are measured and compared to the response to other inotropic interventions (increased both calcium and paired stimulation).

Major Findings: (1) Results indicate that there is no age difference in the dose of ACS necessary to induce ventricular tachycardia in the awake dog. There is no age difference in the toxic threshold to ACS during anesthesia. The peak inotropic response, however, is twofold greater in the adult than in the senescent beagle. This age difference in inotropic response to ACS persisted during beta-blockade. These data are in manuscript form to be submitted for publication.

(2) In isolated rat cardiac muscle, there is a marked significant

decrement in the inotropic response to ouabain, while no age difference exists in response to calcium or paired pacing. The number of studies in cardiac muscle isolated from dogs is too few for analysis at present. There is no age difference in the ouabain inhibition of Na-K ATPase either in the dog or in the rat. This suggests that either the number of receptors or steps intermediate between the receptor and effector are altered with age. These rodent data are in manuscript form to be submitted for publication.

Significance to Biomedical Research and the Program of the Institute: The age difference demonstrated in the direct effect of ouabain on isolated cardiac muscle indicate that the number of sarcolemmal receptor sites or ion exchange pumps may be altered with age. Since cardiac glycosides are widely used in treatment of heart failure, the demonstration of an age difference in response to these agents in several species would be of obvious clinical import.

Proposed Course: (1) To complete the studies in the canine isolated muscle. (2) Investigation of the feasibility of measuring the response to ouabain infusion as a function of age in normal man has begun.

Publications:

Lakatta, E. G.: Perspectives on the Aged Myocardium. In Roberts, J., Adelman, R. C., and Cristofalo, V. J. (Eds.): Advances in Experimental Medicine and Biology. New York, Plenum Publishing Co., 1978, Vol. 97, pp. 147-169.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00025-02 CPB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Prolonged Isometric Relaxation in Cardiac Muscle of the  
Senescent Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. G. Lakatta Chief, Cardiovascular Section CPB, NIA  
H. A. Spurgeon Physiologist CPB, NIA

Other: J. P. Froehlich Medical Officer LMA, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

.8

PROFESSIONAL:

.4

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been demonstrated that isometric relaxation is prolonged in senescent myocardium (Lakatta et al., J. Clin Invest. 55: 61-68, 1975) and that this is accompanied by a diminished rate of Ca<sup>++</sup> accumulation in sarcoplasmic reticulum isolated from the senescent rat heart, when compared to the adult rat heart (Froehlich et al., J. Mol. Cell. Cardiol. 10: 427-438, 1978). The current investigation is designed to determine whether the slow relaxation may be related to hypertrophy of the senescent heart and whether or not relaxation rate may be modified by physical conditioning. In addition, the relationship between mechanical relaxation and sarcoplasmic reticulum function is explored utilizing perturbations of both systems, including chronic thyroid injections and catecholamines and then intracellular messenger equivalents

GRC/CPB-40



## Project Description:

Objectives: In several species ranging from the rat to man, the duration of mechanical isometric relaxation has been demonstrated to be prolonged. To test whether this prolonged mechanical activity is related to hypertrophy observed in the senescent myocardium, adult animals with small hearts were subject to aortic banding to result in a 15-25% increase in heart mass, similar to that seen in senescence. To determine whether physical conditioning may alter the relaxation rate, young and middle aged adult and aged animals were run on treadmill wheels for 4 months prior to study. Prolonged relaxation time could be explained at least in part by a decrement in the ability of the relaxing apparatus to bind calcium in the aged heart. The relaxing apparatus can be isolated in the microsomal fraction and in vitro calcium accumulation measured.

Methods: (1) Microsomes are prepared by the standard method of Hiragawa and Schwartz. Steady state velocity of calcium binding over a range of free calcium concentrations is measured in the presence of oxalate. (2) Mechanical measurements of contraction and relaxation are made in a standard myograph apparatus. These measurements are also made in control young adult and aged hearts, and in hearts of animals subject to aortic banding and in hearts of animals having run on a treadmill at 0.4 MPH, for 30 minutes, 5 days a week for 4 months.

Major Findings: (1) Preliminary results indicate that mild cardiac hypertrophy, induced in middle aged adult rats does not necessarily in itself result in prolonged relaxation or diminished velocity of  $\text{Ca}^{++}$  accumulation in cardiac microsomes. This may indicate that the prolonged relaxation observed in the senescent rat does not solely result from this degree of hypertrophy that accompanies senescence.

(2) The pilot group of studies on rats subject to treadmill running have been concluded and results are currently being analyzed.

Significance to Biomedical Research and the Program of the Institute: The results are significant to biomedical research because they encourage further studies to elucidate the mechanism for the age difference in relaxation. If this can be determined it will extend our knowledge regarding the relaxation process in heart per se. The results are significant to the program of the Institute in that one of the most distinctive features of aged hearts of many species, including man, is a slowing of relaxation. This is of potential clinical significance during times of stress, at high heart rates, when diminished relaxation times could interfere with ventricular filling and lead to enhanced dyspnea and functional impairment.

Proposed Course: (1) The present study measures only steady state rates of calcium accumulation. Our next goal is to measure the rapid burst of activity that precedes the steady state rate, as this may be more tightly coupled to relaxation of the tissue. Additional studies will examine the relationship between these rapid rate kinetics and relaxation in the tissue following manipulations that are known to alter the relaxation process in both systems. These studies will be performed in close collaboration with Dr. Froehlich of the Molecular Biology Section, LMA. Instrumentation to implement these studies is forthcoming.

(2) The feasibility of implementing physical conditioning resulting from daily swimming exercise beginning at a very early age and lasting until pre-senescence (2 mo to 24 mo) will be determined. If feasible, the effect of this conditioning on the relaxation process as well as on many additional age related changes in the cardiovascular system will be investigated.

Publications:

Froehlich, J. P., Lakatta, E. G., Beard, E., Spurgeon, H. A., Weisfeldt, M. L., and Gerstenblith G.: Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. J. Mol. Cell. Cardiol. 10: 427-438, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00026-02 CPB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less)  Age-Associated Alterations in Response to Catecholamines																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">T. Guarnieri</td> <td style="width: 40%;">Clinical Associate (DOD 6/30/78)</td> <td style="width: 20%;">CPB, NIA</td> </tr> <tr> <td></td> <td>E. G. Lakatta</td> <td>Chief, Cardiovascular Section</td> <td>CPB, NIA</td> </tr> <tr> <td></td> <td>C. Filburn</td> <td>Staff Fellow</td> <td>LMA, NIA</td> </tr> <tr> <td></td> <td>H. A. Spurgeon</td> <td>Physiologist</td> <td>CPB, NIA</td> </tr> </table> Other: None			PI:	T. Guarnieri	Clinical Associate (DOD 6/30/78)	CPB, NIA		E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA		C. Filburn	Staff Fellow	LMA, NIA		H. A. Spurgeon	Physiologist	CPB, NIA
PI:	T. Guarnieri	Clinical Associate (DOD 6/30/78)	CPB, NIA															
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA															
	C. Filburn	Staff Fellow	LMA, NIA															
	H. A. Spurgeon	Physiologist	CPB, NIA															
COOPERATING UNITS (if any) F. C. P. Yin, Div. of Cardiology, Dept. of Medicine, Johns Hopkins Medical Institutions																		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch																		
SECTION Cardiovascular Section																		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224																		
TOTAL MANHOURS 1.0	PROFESSIONAL .9	OTHER .1																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (d1) MINORS <input type="checkbox"/> (d2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)  Previous work from this laboratory has demonstrated that when compared to the young adult rat myocardium, that from <u>aged rats</u> exhibits a <u>diminished inotropic response to catecholamines</u> . In addition, the <u>response to these agents in both the canine model and in man is diminished in senescence</u> , compared to that observed in mature adults. The present work includes measurements of <u>cyclic nucleotide levels</u> , and <u>protein kinase activation</u> in perfused rat interventricular septa which have been stimulated with isoproterenol and <u>dibutyryl cAMP</u> , and in which the mechanical response to these agents has been quantitated.																		

GRC/CPB-43



Objectives: (1) To measure the inotropic effect of catecholamines in the perfused interventricular septum of the rat with measurements of cAMP and protein kinase activation in the same tissue. (2) To measure the chronotropic response to catechols in intact dogs. (3) To measure the chronotropic response to catechols in man.

Methods: The rat interventricular septum is cannulated and perfused and force production in a control steady state is measured. The enhancement of force production in response to infused isoproterenol and dibutyryl cAMP is then measured. When the mechanical response has peaked, the septum is quick frozen in liquid nitrogen and analyzed for cAMP, CGMP, protein kinase activity, and protein kinase activation ratio.

Major Findings: The maximum response to catecholamines in the perfused interventricular septum is diminished in senescent when compared to adult hearts, results which are similar to those in isolated papillary muscle (Lakatta et al., Circ. Res., 36: 262-269, 1975). However, neither control levels nor the isoproterenol stimulated levels of cAMP or protein kinase are age related. Likewise the change in the protein kinase activation ration in septa perfused with isoproterenol is not age related. These data and the additional observation that the age difference in mechanical response persists after dibutyryl cAMP indicate that the age difference in mechanical response to catecholamines appears to lie distal to the receptor and distal to protein kinase activation.

Significance to Biomedical Research and the Program of the Institute: The decrease response to all forms of stress - exercise, hypoxia, etc. may be explained at least in part by the diminished response to catecholamines, the physiological mediators of the stress response. Since the diminished stress response is one of the most notable changes that occurs in an aged individual, studies to elucidate the mechanism of the altered response are of major import. These studies require collaboration between molecular biologists and physiologists.

Proposed Course: The response to catecholamines is mediated via a cascade of events, each of which can be isolated and studied. These steps are as follows: (a)  $\beta$ -receptor, (b) cyclic AMP - GMP phosphodiesterase, (c) protein kinase - phosphorylation, and (d) enhancement of calcium flux into the cell. The phosphorylation of sarcoplasmic reticulum and sarcolemma is believed to mediate the enhanced mechanical performance. The feasibility of measuring the protein kinase cAMP phosphorylation and resulting alteration in  $\text{Ca}^{++}$  transport in both isolated preparations of sarcoplasmic reticulum and sarcolemma will be determined. The latter can also be compared in adult and aged hearts by measurements of the catecholamine induced change in the slow inward  $\text{Ca}^{++}$  current, manifested by the catecholamine induced change in the transmembrane action potential.

Publications:

Lakatta, E. G.: Alterations in the cardiovascular system that occur in advanced age. Fed. Proc. (in press).



## PERIOD COVERED

October 1, 1977 to September 30, 1978

## TITLE OF PROJECT (80 characters or less)

Efficacy of Chronic Digoxin Therapy in Stable Congestive Heart Failure

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. L. Fleg Staff Cardiologist CPB, NIA  
E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Other: None

## COOPERATING UNITS (if any)

Div. of Chronic Medicine, Dept. of Medicine, Baltimore City Hosp.  
Div. of Cardiology, Dept. of Medicine, Baltimore City Hosp.

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SECTION

Cardiovascular Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

0.6

## PROFESSIONAL:

0.4

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Despite the nearly universal employment of digitalis in the treatment of chronic congestive heart failure (CHF), there is no objective documentation of its long-term efficacy. The current project is designed to evaluate, in a double blind crossover fashion, the effects of maintenance digoxin therapy in chronic stable CHF, utilizing clinical findings, chest radiography, echocardiography and systolic time intervals to assess baseline left ventricular function non-invasively and maximal exercise testing to determine aerobic work capacity.

GRC/CPB-45

Project Description:

Objectives: For some two hundred years, digitalis glycosides have been the cornerstone of therapy for CHF. The low toxic therapeutic ratio of digitalis results in the development of toxicity in 20-25% of patients and death in up to 20% of patients developing toxicity. Despite this universal employment of digitalis in the treatment of CHF, there is no objective documentation of its long-term efficacy. The current project utilizes several non-invasive parameters of cardiac function to assess the effects of maintenance digoxin therapy in stable CHF in a double blind crossover manner.

Methods: Patients in the Baltimore City Hospital Cardiology Clinic and the Chronic Hospital who have been on the same CHF medical regimen for at least 3 months will be studied. After a baseline clinical history and physical exam, each patient will receive a resting echocardiogram, systolic time intervals (STI), chest x-ray, and when possible, a maximal stress test. He will then be randomly assigned in a double blind fashion to either digoxin or placebo for 3 months and the studies repeated at the end of this period. Crossover will then occur and the testing repeated again 3 months later.

Major Findings: Thus far, none of the patients withdrawn from digitalis has required urgent reinstituting of digoxin. Detailed analysis of the various tests is currently being implemented.

Significance to Biomedical Research and the Program of the Institute: If maintenance digitalis can be discontinued without ill effect in a sizable percentage of patients with stable chronic CHF, many episodes of toxicity (and death) could be avoided and medical expenses reduced. It should be noted that a sizable portion if not the majority of persons in whom digitalis preparations are prescribed are over 60 years of age.

Proposed Course: These non-invasive techniques for assessing cardiac function will continue to provide valuable objective parameters for evaluating the response to digitalis therapy in cardiac patients.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00011-06 CPB																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less) Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">E. M. Dax</td> <td style="width: 30%;">Visiting Associate</td> <td style="width: 10%;">CPB NIA</td> </tr> <tr> <td></td> <td>T. M. Kelly</td> <td>Clinical Associate (EOD, July 1, 1977)</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>M. S. Katz</td> <td>Clinical Associate (resigned June 30, 1977)</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>R. I. Gregerman</td> <td>Chief, Endocrinology Section</td> <td></td> </tr> </table> OTHER: None			PI:	E. M. Dax	Visiting Associate	CPB NIA		T. M. Kelly	Clinical Associate (EOD, July 1, 1977)	CPB NIA		M. S. Katz	Clinical Associate (resigned June 30, 1977)	CPB NIA		R. I. Gregerman	Chief, Endocrinology Section	
PI:	E. M. Dax	Visiting Associate	CPB NIA															
	T. M. Kelly	Clinical Associate (EOD, July 1, 1977)	CPB NIA															
	M. S. Katz	Clinical Associate (resigned June 30, 1977)	CPB NIA															
	R. I. Gregerman	Chief, Endocrinology Section																
COOPERATING UNITS (if any) Department of Surgery Baltimore City Hospitals																		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch																		
SECTION Endocrinology Section																		
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224																		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1.0																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)  These studies deal with influences of <u>age</u> on the biochemistry of <u>hormone-sensitive adenylate cyclases</u> in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of hormone responsiveness and biological <u>membranes</u> , with special emphasis on the relationship between adenylate cyclase and <u>hormone receptors</u> .																		

GRC/CPB-47

Objectives: Hormone action involves the following steps: a) interaction of the hormone with a hormone-specific chemical entity generally termed a "receptor" b) initiation of another biochemical reaction c) expression of the effect by alteration of cell function. Quantitation of hormone receptors has become possible in the last few years, while the subsequent steps have been under investigation for about a decade. After interaction with receptors some hormones exert their action by activation of the enzyme adenylate cyclase (AC) and the subsequent production of adenosine-3'-5' monophosphate (cAMP) from ATP. Many cell functions are under control of cAMP. Aging is known from many studies to be associated with altered hormonal responsiveness, but the precise mechanisms have only recently come under study. Our investigations hope to elucidate this area through quantitation of receptor number, exploration of factors regulating receptor number, control of relationships between receptors and adenylate cyclase ("coupling"), regulation of adenylate cyclase, and elucidation of alterations beyond cAMP. Both the hormone receptors related to adenylate cyclase and the enzyme itself are integral parts of the cell's outer membrane. Our work, therefore, is in a real sense a study of the effects of aging on cell membranes. Changes of epinephrine, glucagon and ACTH sensitive adenylate cyclases have been previously described in this laboratory during aging and dietary manipulations.

Methods employed: Tissues used are from experimental animals (mainly the rat) and from man (surgical or necropsy specimens). Tissue homogenates, isolated cells, and cell membranes are employed, cells being isolated by collagenase digestion and membranes by density gradient centrifugation, etc. Adenylate cyclase is quantitated by a labeled substrate assay in which  $\alpha$ -<sup>32</sup>P ATP is converted to  $\alpha$ -<sup>32</sup>-cAMP and the latter quantitated after isolation on columns. Use of two marker isotopes (<sup>3</sup>H-cAMP and <sup>14</sup>C-cAMP) enables precise correction of losses due to destruction during incubation on chromatography. Receptor quantitation utilizes labeled hormones or, for technical reasons, labeled hormone antagonists. Protein factors are isolated by standard techniques of protein fractionation (gel and ion-exchange chromatography, etc.).

Major Findings: 1) Cytosol factors required for adenylate cyclase activity. We previously noted an increase of epinephrine sensitive adenylate cyclase in rat liver during aging. During that work, loss of epinephrine and glucagon-sensitive adenylate cyclases activity was noted during preparation of membranes from crude homogenates. The mechanism of loss is partly due to the removal of GTP but also to removal of one or more proteins which interact with the membranes to stimulate hormone-sensitive cyclase. These proteins have been partially characterized and a technique for their quantitation developed. This aspect of the work has been submitted for publication. Further work on the isolation of the factor(s) is progressing. Purification of the glucagon factors has been achieved through ammonium sulfate precipitation and chromatography on Sephadex G-100. At least two glucagon factors appear to be present. Parallel work is in progress with the epinephrine factor. Eventual isolation of these proteins should help elucidate the nature of the adenylate cyclase complex and the character of the age-related changes of cyclase activity.



2) Concentration of  $\beta$  Receptors in Rat Liver and Fat. The concentration of  $\beta$  adrenergic receptors in liver cells of young animals is very much less than in other systems studied ( $\sim 30$  fmole/mg membrane protein vs  $\sim 600$  fmole/mg in fat). Extensive attempts to quantitate the receptor in adult animals have been made using two antagonist ligands,  $^{125}$ I-hydroxybenzylpindolol and  $^3$ H-dihydroalprenolol (DHA), and an agonist,  $^3$ H-hydroxybenzylisoproterenol. Although these ligands appear to measure  $\beta$  receptors, i.e. binding sites which can be abolished with antagonist loading, the sites are not, in our hands, stereospecific. Since the receptors, as judged by adenylate cyclase activation, are stereospecific for agonists and antagonists, we must conclude that it has not been possible to quantitate the  $\beta$  receptors in liver. Although we continue in our efforts along these lines, we must question reports purporting to relate  $\beta$  receptors of liver to biochemical phenomena. In many studies, the criterion of stereospecificity has not been applied and the results are therefore suspect. A paper dealing with these problems is in preparation.

No difficulty has been encountered in quantitating  $\beta$  receptors in fat cells with DHA. The concentration is  $\sim 600$  fmoles/mg membrane protein. Preliminary results suggest altered receptor affinity and a "compensatory" increase of receptor number. These results open the way to studying the interrelationships of receptor number, hormone sensitive adenylate cyclase and the known age-related decrease of lipolysis.

3) Age-Related Alterations of Fat Cell Membranes. In our previous studies, most of the age-related change of hormone-sensitive adenylate cyclase appears to be maturational, i.e., the largest changes are between the period of active growth (1-2 months) and adulthood (6-12 months) rather than between the latter and senescence (24 months). We now have evidence that the membranes from young animals are qualitatively different for those of older animals. The concentration of NaCl required for maximal stimulation of 2 mo membranes is 50-100 mM while 200 mM salt is needed for older membranes. Moreover, salt enhances the effect of GTP to a much greater extent and in a different dose-response fashion in young than in old animals. GTP alone is stimulatory for 2 mo membranes but has no effect on material from older animals. These biochemical studies suggest age-related structural changes in membranes and complement our earlier observation on membrane instability during aging of rat liver (see Publications).

4) Anion, Cation and GTP Effects on Adenylate Cyclase. Previous work from this laboratory has described anion stimulation of adenylate cyclase of liver and fat. These studies have now been extended to include cations and their interactions. New and rather complex patterns emerge in which both charged species are shown to be important. Some effects previously attributed to  $Mg^{2+}$  are now shown to be anion related. Anions and cations also affect GTP action on membranes. The results have been prepared for publication.

Significance to Biological Research and the Program of the Institute. Our ongoing studies are producing basic information upon which depends on our understanding of age-related changes of hormone sensitivity. In addition,

new insights are being obtained concerning the biochemistry of receptors and adenylate cyclase.

Proposed Course of the Project: Purification of cytosol protein activators of adenylate cyclase will be continued. The problem of  $\beta$  receptor quantitation will be resolved. Assays for  $\alpha$  receptors will be developed to allow study of the  $\alpha - \beta$  interrelationships. Dietary and hormonal manipulations will explore the stability of the receptors and adenylate cyclase and the dependence on age. An effort will be made to identify the biochemical locus at which aging produces impaired lipolysis.

Publications:

Kalish, M. I., Katz, M. S., Pineyro, M. A. and Gregerman, R. I.: Epinephrine- and glucagon-sensitive adenylate cyclases of rat liver during aging. Evidence for membrane instability associated with increased enzymatic activity. Biochim. Biophys. Acta 483: 452-466, 1977.

Katz, M. S., Kalish, M. I., Pineyro, M. A. and Gregerman, R. I.: Quantitation of epinephrine- and glucagon-sensitive adenylate cyclases of rat liver. Implications of alterations of enzymatic activities during preparation of particulate fractions and membranes. Biochim. Biophys. Acta 540: 205-220, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00012-06 CPB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. S. Roth	Research Chemist CPB NIA
OTHER:	W. C. Chang R. I. Gregerman	Visiting Fellow CPB NIA Chief, Endocrinology Section CPB NIA
COOPERATING UNITS (if any)  <div style="display: flex; justify-content: space-between;"> <span>D. D. Schocken</span> <span>Cardiology Fellow</span> <span>Duke Univ. Sch. of Medicine</span> </div>		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Endocrinology Section		
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	, 2.1	0.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <span><input type="checkbox"/> (a) HUMAN SUBJECTS</span> <span><input checked="" type="checkbox"/> (b) HUMAN TISSUES</span> <span><input type="checkbox"/> (c) NEITHER</span> </div> <div style="display: flex; justify-content: space-between;"> <span><input type="checkbox"/> (a1) MINORS</span> <span><input type="checkbox"/> (a2) INTERVIEWS</span> </div>		
SUMMARY OF WORK (200 words or less - underline keywords)  This project is mainly involved in relating <u>age</u> , the <u>intracellular</u> and <u>cell surface receptors</u> for <u>hormones</u> and the biological responsiveness of hormone-sensitive tissues.		

GRC/CPB-51

## Project Description:

Objectives: This project attempts to elucidate the mechanisms by which the actions of hormones are altered during aging.

Methods Employed: Whole animals, isolated tissues, and defined cell populations in short term culture are used. In addition, white blood cells are obtained from Longitudinal Study participants in accordance with Human Research Committee guidelines. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Affinity chromatography is used to isolate hormone receptors for measurement of synthetic and degradative rates as well as purification for preparation of antisera and immunochemical titration. Hormonal control of various cellular metabolic processes such as nutrient transport and utilization are measured by standardized techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) We have previously reported that various physiological functions mediated through intracellular glucocorticoid receptors are altered during aging and that receptor concentrations are concomitantly reduced. Glucocorticoid receptors have now been partially purified from cerebral cortex and adipocytes of rats of various ages. Such receptors bind specifically to dexamethasone-Sepharose beads and can be eluted under rigidly defined conditions of temperature, pH and ionic strength. Eluted material has been characterized physiochemically by density gradient sedimentation and gel electrophoresis. Under low ionic strength conditions receptors sediment at approximately 8S with an apparent molecular weight of about 100,000. At higher ionic strength sedimentation coefficients are approximately 4S, suggesting dissociation of receptor subunits. Antisera have been prepared against these proteins in rabbits and are capable of detecting glucocorticoid receptors by immunoprecipitation. These techniques have been used to measure the rates of receptor biosynthesis in isolated adipocytes which are labeled with <sup>3</sup>H-amino acids for varying periods of time. Preliminary observations reported last year have been confirmed. The rate of receptor synthesis is 60-70% lower in adipocytes from senescent (24-26 mo.) rats than in cells from mature (12 mo.) counterparts. Overall protein synthesis and amino acid uptake do not appear to be altered, however. These findings are consistent with a previously observed 60-70% reduction in overall glucocorticoid receptor concentration and the idea that key regulatory proteins may be selectively affected during aging.

An epididymal fat pad explant system has also been established. Explants can be cultured for several days with cells remaining responsive to glucocorticoid hormone control of energy metabolism. This system should prove useful for metabolic manipulations in vitro.

2) In addition to changes in intracellular components of hormone action, studies of age changes in the cell membrane and its receptors have also been performed. With increasing age the ability of the rat adipocyte



membrane glucose transport system to be regulated by various agents is almost completely lost. Such substances include insulin, glucocorticoids, norepinephrine, vitamin K<sub>5</sub> and hydrogen peroxide. With the exception of norepinephrine these compounds reduce the level of glucose transported into the cell by various mechanisms. Thus, the membrane may become generally refractory to this type of regulation during aging. In contrast, norepinephrine stimulates glucose transport and metabolism. We have observed that such stimulation can be prevented by specific  $\alpha$ , but not  $\beta$ , adrenergic antagonists, and thus appears to be mediated thru  $\alpha$  adrenergic receptors. Preliminary studies have detected these receptors on adipocyte membranes with the use of the labeled  $\alpha$  antagonists dihydroergocryptine and WB 4101 and with norepinephrine.

Concentrations of other specific membrane hormone receptors also appear to be reduced during aging. Dopamine receptor levels of rat corpus striatum are decreased by 35% between 6 and 24 months of age. No change in binding affinity is observed, however. Such reductions may be responsible for the age related loss of dopamine stimulation of brain adenylate cyclase reported by many investigators.

Control of cardiac  $\beta$ -adrenergic receptors by thyroid hormones in rats of various ages has been examined. Caution must be exercised in quantitating receptors in such studies since the relative contamination of the sarcolemmal fraction by non-membrane proteins may also be affected by thyroid hormone treatment. Preliminary results suggest that control of cardiac  $\beta$ -adrenergic receptors during aging and/or by thyroid hormones may be related to altered contractile responsiveness, but the physiological and biochemical significance of such regulation remains unclear.

Finally, studies on adrenergic receptors and responsiveness in human peripheral lymphocytes have been continued. A class of  $\beta$ -adrenergic binding sites with higher affinity in lower capacity than that reported previously has been identified by several investigators and confirmed in our laboratory. Preliminary results suggest that this species is also reduced in concentration during aging.

#### Significance to Biomedical Research and the Program of the Institute:

Altered responsiveness to hormones is characteristic of a generalized functional decline during aging. Elucidation of those mechanisms by which hormone actions change with age is essential to any understanding of senescence. Accumulation of such basic information may allow prevention or reverse some effects of aging by appropriate, endocrinological, pharmacological or biochemical intervention.

Proposed Course of the Project: 1) The molecular and possibly neuroendocrine mechanisms involved in the age associated loss of glucocorticoid receptors and responsiveness in rat adipocytes and brain will be further examined. Rates of receptor turnover as well as synthesis will be measured during aging. Specific inhibitors of RNA and protein metabolism will be employed to localize the molecular lesions responsible for reduced biosynthetic rates in old cells. Attempts to regulate receptor levels using insulin, indomethacin and

several related agents will also be made. Glucocorticoid receptor antisera will be tested for sensitivity and specificity, and if necessary further purified by immunoadsorption. Specific antisera will then be used to determine whether non-functional, but still immunoreactive receptors can be detected in senescent cells.

Epididymal fat pad explants will be cultured in the presence of serum factors from rats of various ages to determine whether any humoral factors may be involved in changes in glucocorticoid responsiveness. Parallel experiments will be attempted in vivo using explants in nucleopore envelopes or direct transplantations.

2) Studies of cell membrane receptors and their control during aging will be continued.  $\alpha$  adrenergic receptors of rat adipocytes will be examined during aging to determine whether changes at this level are related to the altered  $\alpha$  adrenergic control of glucose metabolism.  $\beta$ -adrenergic receptors will be further characterized in circulating lymphocytes from longitudinal study subjects. Correlation of certain biological responses with receptor levels will also be attempted. In addition, the possibility of separating platelets from the same peripheral blood samples for use in  $\alpha$  adrenergic responsiveness and receptor studies by Dr. Schocken at Duke University is also under consideration.

Clarification of the role of  $\beta$ -adrenergic receptor regulation in age related cardiac responsiveness changes will be continued. Thyroid hormones, 6-hydroxydopamine and possibly exercise will be utilized to regulate the levels of these receptors.

#### Publications:

Roth, G. S.: Hormonal Receptor and Responsiveness Changes During Aging. In Bergsma, D. and Harrison, D. H. (Eds.): The Genetics of Aging. Original Article Series of the National Foundation. 1978, Vol. XIV, p. 365-384.

Roth, G. S., Chang, W-C. and Gesell, M. S.: Changes in Hormone Receptors During Aging: Role in Altered Hormonal Responsiveness. In Vassileva-Popova, J. (Ed.): Second International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Aging, New York, Plenum Press, in press.

Roth, G. S.: Hormone Action During Aging: Alterations and Mechanisms. Mechanisms in Aging and Development, in press.

Joseph, J. A., Berger, R. E., Engel, B. T. and Roth, G. S.: Age Related Changes in the Nigrostriatum: A Behavioral and Biochemical Analysis. Journal of Gerontology, in press.

Roth, G. S.: Changes in the Action of Hormones and Their Receptors During Aging. In Adelman, R. C. and Smith, L. (Eds.): NIA Workshop on the Biochemistry of Aging, in press.

Roth, G. S.: Hormone Receptor Changes During Aging. In Melnechuk, T. (Ed.): Receptor Disorders, in press

Roth, G. S.: Receptor Changes and the Control of Hormone Action During Aging. In King, D. W. (Ed.): Given Institute of Pathology Seminar on Aging, in press

Roth, G. S.: Hormone Receptor Changes During Adulthood and Senescence: Significance for Aging Research. Federation Proceedings, in press.

Schocken, D. D. and Roth, G. S.: Age Associated Alterations in Adreno-receptor. In Current Concepts in Adrenoreceptors, Amsterdam, Elsevier/North Holland, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00013-04 CPB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Hormones, hormone receptors, and aging. III. Aging and the human male reproductive system.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	S. M. Harman	Senior Investigator CPB NIA
OTHER:	P. D. Tsitouras C. E. Martin R. I. Gregerman	Visiting Associate Senior Investigator Chief, Endocrinology Section CPB NIA CPB NIA CPB NIA
COOPERATING UNITS (if any) None		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Endocrinology Section and Human Performance Section		
INSTITUTE AND LOCATION NIA, GRC, Baltimore, Maryland 21224		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  A series of tests of <u>reproductive endocrine function</u> are being carried out on male volunteers in the <u>Baltimore Longitudinal Study on Aging</u> . Cross sectional and longitudinal data have been obtained which deal with testosterone and estrogen levels in plasma. <u>Pituitary function</u> with LHRH and plasma <u>pituitary gonadotrophins</u> is being assessed. Level of <u>sexual activity</u> is being related to the hormone assays.		

GRC/CPB-56



## Project Description:

### Objectives:

A. Background - Steroid hormones secreted by the gonad play an important role in regulating body economy throughout the lifespan. In the male testosterone, the major testicular steroid hormone, not only maintains secondary sex characteristics and sexual function, but is responsible for positive nitrogen balance and maintenance of increased muscle mass, for skeletal integrity, and possibly for such diverse functions as rate of healing and general level of aggressiveness. Steroid secretion by the testis is maintained by the pituitary hormone, LH, which is itself regulated by hypothalamic secretion of another hormone LHRH. This intricate control mechanism is in turn inhibited by rising plasma levels of testosterone, forming a complete system with its own feedback device to assure constancy of function. A second pituitary hormone, FSH, also under hypothalamic regulation by LHRH, controls testicular production of germ cells.

B. Current knowledge - Alteration of function of the male reproductive system with advancing age has been reported by a number of investigators. Notable changes described include a decrease in the circulating level of testosterone after age 60, an increase in plasma protein binding of testosterone, which further lowers the level of "free" (and thus, presumably, bio-available) testosterone, and an increase in circulating levels of female hormones (estrone and estradiol). At the same time the plasma FSH and LH levels have been found to increase, suggesting a primary failure of the testis, which releases the pituitary from feedback inhibition control. Some evidence also exists for a reduction in pituitary function with age in that response of the pituitary to exogenous LHRH appears to be somewhat reduced. Since study populations have not been well-defined in terms of such variables which may affect hormone secretion such as nutrition and obesity, alcohol and tobacco consumption, and health and general level of activity it is difficult to interpret the significance of currently available data to aging, per se. Furthermore, none of the available studies has a longitudinal design, which makes their interpretation subject to all of the difficulties which characterize cross-sectional aging studies. None of the available studies have attempted to correlate changes in sex hormone regulation with such variables as sexual behavior, prostate disease, cardiovascular disease, or subtle measures of psychological function.

C. Present study - Using a well-characterized group of men from the Baltimore Longitudinal study on Aging, the effects of age on testicular function and pituitary-gonadal regulation independent of intercurrent illness, excess alcohol consumption, obesity, etc. is being defined. In addition, an attempt will be made to correlate altered function with the above variables and with changes in libido, coronary disease, and prostate disease.

Methods employed: (1) Plasma gonadotrophins are being assayed using a double antibody radioimmunoassay. (2) Plasma testosterone and dihydrotestosterone are measured with a very precise recently developed radioimmunoassay. (3)

Plasma estrone and estradiol are assayed using a charcoal radioimmunoassay method. (4) Semen analysis has been performed by standard techniques of counting and staining. (5) The fraction of free testosterone in plasma is being estimated by an ion-exchange column method developed in our laboratory. (6) Blood samples are obtained from a healthy, non-obese subgroup of the BLS population before and after intravenous injection of 100 µg of LHRH to test for pituitary gonadotrophin reserve and then after intramuscular injection of human chorionic gonadotrophin to test for testis secretory reserve. (7) Freeze-dried plasma samples from the BLS subjects, taken in previous years, will be used to survey relationships between testosterone level and sexual function in collaboration with Dr. C. Martin. (8) Computerized techniques for regression and multiple regression analysis will be used to evaluate data for associations of altered sex hormone secretion with other variables. (9) Subjects will be restudied at 4 year intervals to provide longitudinal data. Four cycles of study are anticipated.

Major Findings: 76 subjects over the age range 25 to 89 have participated to date. There are a minimum of 10 subjects in each decade. Due to a shortage of young BLS participants, it was necessary to recruit a small number of paid volunteers (age 25-29) of comparable social and educational background.

Data show a significant increase in the basal FSH and LH levels with age, but, unexpectedly, no apparent change in total resting testosterone levels over the age range studied. These data are in contrast to data from several other studies showing a significant decline in mean testosterone with age. This combination of findings suggests that our subjects do experience some failure of testicular secretory mechanisms which is compensated for by the increased output of gonadotrophins, whereas other workers have found a more severe and uncompensated failure in a significant number of their subjects. Our study also differs from previous reports in detecting no alteration of plasma estrogens or dihydrotestosterone with age, both of which are reported to increase in other studies. A likely explanation for differences between our findings and those of others may lie in non-comparability of populations investigated with regard to obesity, use of alcohol, chronic disease, and other similar variables, especially since older subjects from other studies have been recruited from clinic and old-age home populations. Information on possible changes in protein binding of sex steroid hormones with age in our study group is presently being obtained. Preliminary analysis of testis secretory response to hCG suggests a diminished testicular reserve with age, but confirmation of this finding awaits more sophisticated computer analysis.

Pituitary function testing with LHRH shows no decrement in the absolute response of the pituitary for LH or for FSH in terms of peak or integrated (area under the secretion curve vs. time) response above baseline. A more common criterion for this type of testing is the relative response (peak or increment divided by the basal), since basal levels do affect the peak and the increment. Using this criterion there is a definite decrease in pituitary response to LHRH both for LH and for FSH. When a subgroup of young and old subjects matched for basal levels of LH are tested for absolute (peak or integrated) response differences, a difference between young and old groups is quite apparent, suggesting that there is a real alteration in pituitary

gonadotrophic function with age.

Additional findings include a small but significant age-related decrease in testicular size. Sperm analyses have been hampered by willingness of only about one-third of subjects tested to comply with requests for sperm samples. Results do suggest that in our population the absolute number of sperm ejaculated does not decrease, but there is a trend toward increasing numbers of immature spermatozoa appearing in ejaculates with age.

There does not appear to be any correlation of reported sexual activity levels and basal plasma testosterone in the 76 subjects studied to date.

#### Significance to Biomedical Research and the Program of the Institute:

Correlation of changes in sex hormones with behavioral, physiological or pathological status with age may shed light on mechanisms of various changes which have been observed in the aging male, including decreased libido and impotence, prostatic hyperplasia, and increased incidence of coronary disease (compared with the female). Normative data on levels of hormone secretion may help differentiate men with underlying disease processes from those who are simply aging.

Proposed Course: The first cycle of this study is now complete and a new cycle begins in the summer of 1979. Compilation of data from the first cycle for computerized analysis is underway and may lend some insights not apparent from the preliminary analysis. Further work needs to be done in defining the effects of alcohol, obesity, tobacco, level of physical activity, and various chronic illnesses on the testosterone level. This will necessitate the study of a much larger group than heretofore, but in a more limited way (testosterone assay only). In collaboration with Dr. Martin a large group (180) of subjects with high, medium or low levels of sexual performance will have stored samples analyzed to determine whether, in this more complete group, any correlation of hormone level and libido exists. In addition a collaborative study is planned with Dr. M. Blackman of City Hospitals to examine the secretory pattern of pituitary hormone subunits in our first cycle subjects. This investigation may detect altered patterns of protein synthesis and assembly with age. Finally, an investigation needs to be undertaken as to how well a testosterone level taken at a certain time of day or on a particular day characterizes a particular man at other times of day (i.e., diurnal variation) or for other days, and whether age affects the pattern of such variations. Such data are crucial to the interpretation of our BLS results.

#### Publications:

Harman, S. M. and Danner, R. L.: Rapid measurement of an index of testosterone binding to serum binding globulins using ion exchange columns. J. Clin. Endocrinol. Metab. 45: 953-959, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00023-03 CPB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors, and Aging, IV. Aging and Leydig cell function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. M. Harman Senior Investigator CPB NIA

OTHER: P. D. Tsitouras Visiting Associate CPB NIA  
G. S. Roth Research Chemist CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The function of Leydig cells isolated from rat testis is studied in vitro in comparisons of old and young rats. Testosterone production, cell number of gonadotrophin receptors, cAMP production are assayed in order to localize the age-related alterations of Leydig cell function.

GRC/CPB-60



Objectives: The Leydig cells of the testis secrete the major male hormone, testosterone, which is essential to normal male development and function, and which also has important actions at sites as diverse as bone, muscle, skin, and central nervous system. A number of studies have shown reduced function of Leydig cells both in animals and in man. Leydig cell function is normally under the control of the pituitary hormone, LH, which interacts with membrane receptors to activate adenylate cyclase. The resultant increase in cAMP leads to catalytic phosphorylation and activation of a system of protein kinases, which in turn alter the cell's internal metabolism and lead to hormone production and secretion. The goal of the present study is to define and investigate the nature of the defect appearing in an animal Leydig cell system with advancing age.

Methods employed: Matched pairs of young (4-9 months) and old (22-26 months) rats from the GRC Wistar colony are killed by cervical dislocation and the testis removed and partially digested with collagenase. Tubular and interstitial elements are then separated by filtration, and, for cAMP experiments, Leydig cells are further purified by density gradient centrifugation. The number of viable Leydig cells in each preparation is estimated using a histochemical (3- $\beta$ -hydroxy-dehydrogenase) stain and the trypan blue exclusion technique. Short term incubations are then carried out with varying doses of human chorionic gonadotrophin (hCG, an LH-like hormone) to determine cell production of testosterone, cAMP, or cell membrane binding capacity and affinity for radioactively labelled hCG. Testosterone secreted into the medium is analyzed by radioimmunoassay using Florisil for separation of bound and free hormone. cAMP present in washed cells (total) and bound to intracellular protein (presumably protein phosphokinase) are estimated by standard techniques and radioimmunoassay. Membrane receptor capacity is estimated from Scatchard plots obtained from incubations of Leydig cell membranes with labelled hCG.

Major findings: It has become apparent that, although the weight of the testes of older animals is comparable to that of younger rats, and the number of Leydig cells recovered is similar or even slightly increased in the old animals, there is a significant (50%) reduction in testosterone secretory capacity per  $10^6$  Leydig cells as determined from dose-response curves to hCG at various incubation times. There is also a significant (30%) reduction in the number of hCG binding sites on cell membranes with age. Since occupancy of only 15-20% of receptors is necessary for maximal hormone secretion by the cells, it is improbable that the reduction in testosterone secretion is caused by a loss of receptor number. Receptor affinity does not appear to be altered with age. Further evidence that the major defect does not lie at the level of hormone-receptor interaction comes from observation that, in our preparations, there is no age-related difference in the hCG-induced increment in cytoplasmic or protein bound cAMP, a step which is distal to the hormone-receptor and, indeed, the receptor-cyclase interactions.

Significance to Biomedical Research and the Program of the Institute:

If the nature of aging is to be understood, the precise biochemical defects in the function of differentiated cells of aging animals as well as the

defects hindering the replacement of such cells from populations of less differentiated cells must be investigated. Since the characteristic response pattern and details of many of the intermediate steps in the metabolism of Leydig cells is known, and since an aging defect in the function of these cells can be demonstrated and probably has broad physiologic significance, this model system seems to be particularly suited for the study of aging processes.

Proposed Course: Continuing investigation of the function of the aged Leydig cell will proceed in two directions. First, since we have previously demonstrated in vivo, in old rats, evidence of gonadotrophin induced repair of testis secretory function. The nature of this repair (whether recruitment of new cells, or increased secretion by cells already differentiated, etc.) will be investigated. Second, work will be continued to try to define the defect found in the aged Leydig cells with regard to testosterone production. Likely areas for investigation include measurement of rate-limiting enzymes in the steroid secretory pathway, cellular energy producing systems and mitochondrial function, and role of the system for generating reduced pyridine nucleotides essential for hormone production.

Publications:

Harman, S. M., Danner, R. L. and Roth, G. S.: Testosterone Secretion in the Rat in Response to Chorionic Gonadotrophin: Alterations with Age. Endocrinology 102: 540-544, 1978.

SMITHKIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00014-08 CPB
PERIOD COVERED <b>October 1, 1977 to September 30, 1978</b>		
TITLE OF PROJECT (80 characters or less)  <b>The Biochemistry of renin and renin substrate.</b>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: G. Pourmotabbed  H. J. Chou  R. I. Gregerman	Visiting Associate (EOD 8-1-77)  Visiting Fellow (resigned, 10-1-77)  Chief, Endocrinology Section	CPB, NIA  CPB, NIA  CPB, NIA
COOPERATING UNITS (if any)  <b>J. H. Shaper, Department of Oncology, Johns Hopkins University Hospital</b>		
LAB/BRANCH <b>Gerontology Research Center, Clinical Physiology Branch</b>		
SECTION <b>Endocrinology Section</b>		
INSTITUTE AND LOCATION <b>NIA, GRC, Baltimore, Maryland 21224</b>		
TOTAL MANYEARS: <b>1.2</b>	PROFESSIONAL: <b>1.2</b>	OTHER: <b>0</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <b>This project explores the biochemistry of <u>renin</u> and <u>renin-substrate</u>, proteins involved in hypertension. The substrate is being purified and labeled in order to develop a new assay for renin. Other studies include chemical modification of the substrate, isolate tion of <u>renin inhibitors</u>.</b>		

GRC/CPB-63

## Project Description:

Objectives: The enzyme renin, produced and secreted from specialized cells of the renal glomerulus, appear to be involved in several varieties of hypertension. Secretion of the enzyme is under control of a variety of physiologic and pathophysiologic factors, e.g., salt restriction, volume depletion, sympathetic nervous system control. In the circulation the enzyme acts on a plasma glycoprotein to release the decapeptide, angiotensin I, which is then activated by C-terminal cleavage to a smaller octapeptide, angiotensin II, in the lung and other tissues. Angiotensin II in turn influences the secretion of aldosterone, principal mineralocorticoid hormone of the adrenal, and has other direct effects on the cardiovascular and central nervous systems. In certain pathologic states renin has a direct role in the pathogenesis of hypertension, as it does in renovascular stenosis. Recent work suggests that renin may be more directly involved in common or "essential" hypertension. Preliminary reports indicate that an inhibitor of the angiotensin converting enzyme, i.e., prevention of angiotensin II formation, results in amelioration of hypertension in many patients. Finally, the secretion of renin (and aldosterone) is markedly influenced by aging in man, while hypertension is an age-dependent disease.

Progress in these areas has depended on advances in our knowledge of the basic biochemistry of renin and its substrate. Our own laboratory has been involved in the development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay and by immunoassays of angiotensin I. Renin substrate has been purified from porcine and human plasma by column chromatography on DEAE cellulose, Con-A Sepharose and Sephadex G-100. Other techniques are standard biochemical methods (amino acid analysis, high voltage electrophoresis, etc.).

Major Findings: I. Separation of Human Renal Renin and Pseudorenin by Affinity Chromatography on Hemoglobin-Sepharose-2B. It has been known for some time that human renin and plasma contain pseudorenin, a renin-like enzyme. In purified systems pseudorenin forms angiotensin I, while in plasma the enzyme is inactive toward protein renin substrate, presumably because of the presence of inhibitors. We have now perfected a simple affinity chromatography method (hemoglobin-Sepharose-2B) which separates human renal renin from pseudorenin. The latter material behaves like cathepsin D, a ubiquitous lysosomal acid hydrolase. This work has now been published, and our suggestion that pseudorenin is cathepsin D has been confirmed by two recent publications from other laboratories

II. Isolation of a Pseudorenin Inhibitor. During our purification of renin substrate, we isolated a small molecular weight, heat stable material, which is an inhibitor of plasma pseudorenin, now better termed cathepsin D. The significance of plasma levels of cathepsin D remain to be assessed. However



cathepsin D has been recently implicated in some demyelinating diseases and some efforts to affect these processes is being made using pepstatin, a potent but non-specific cathepsin D inhibitor. We hope to characterize our inhibitor further in the hope of elucidating its chemistry, specificity, and possible physiology.

III. Polymeric Inhibitor of Renin. We have previously described the synthesis of a dextran-pepstatin conjugate which inhibits renin and pepsin. The usefulness of this material in vivo has not been assessed as yet, but we would expect it to have prolonged action. The work has now been published in preliminary form and is being prepared for publication in detail elsewhere.

IV. Chemistry of Renin Substrate. In our last annual report we described the purification of hog renin substrate. A number of experiments also suggested that treatment of human renin substrate with dithioerythritol (DTT, Cleland's reagent) would render it susceptible to attack by the hog enzyme to which it is ordinarily resistant. Later work did not confirm these earlier results. It has, however, been shown that DTT treated hog substrate is more rapidly attacked by homologous renin. The kinetics and biochemistry of this phenomenon remain to be completed and are in progress. Further work on the large-scale isolation of renin substrate was suspended due to the resignation of one of the principal investigators.

IV. Labeling of Renin Substrate. Development of a direct labeled substrate assay for renin has been a long term goal of this and other laboratories. To this end we have, as previously described, purified renin substrate in order to label the material with a relatively specific N-terminal (<sup>125</sup>I-Bolton-Hunter) reagent. Labeled angiotensin I carrier was prepared and its extraction characteristics determined. Model labeling experiments with insulin and albumin have been completed. It would appear that the renin substrate can now be labeled to sufficient specific activity; this stage is about to begin.

Significance to Bio-medical Research and the Program of the Institute. Ongoing work should allow elucidation of new classes of natural and synthetic inhibitors of renin and cathepsin. These agents may help clarify the nature of the hypertensive states and their biochemical bases. A new, direct assay of renin is being fashioned. If successful, this methodology should be useful for diagnostic applications.

Proposed Course of the Project. We propose to characterize the plasma cathepsin inhibitor. Labeled substrate for the new assay will be synthesized.

#### Publications:

1. Chou, H.J., and Gregerman, R.I.: Preparation and Characterization of a Dextran-Pepstatin Conjugate, A New and Potent Inhibitor of Renin and Pepsin. In Goodman, M. and Melenhofer, J. (Eds.): Peptides-Proceedings of the Fifth American Peptide Symposium. John Wiley and Sons, Inc., 1977, 213-214
2. Chou, H.J., Shaper, J.H. and Gregerman, R.I.: Separation of Human Renal Renin and Pseudorenin by Affinity Chromatography on Hemoglobin-Sepharose-2B. Biochimica et Biophysica Acta 524: 183-187, 1978.

Chou, H. J., Rehfield, P. L. and Gregerman, R. I.: Linkage of the N-terminal peptide portion of renin substrate to the remainder of the protein. Evidence for an alkali labile Tyr-Ser and against an ester bond. Biochim. Biophys. Acta (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00015-20 CPB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (60 characters or less)  The Baltimore Longitudinal Study of Human Aging														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">R. Andres</td> <td style="width: 35%;">Chief, Clinical Physiology Branch</td> <td style="width: 15%;">CPB NIA</td> </tr> <tr> <td></td> <td>A. H. Norris</td> <td>Chief, Human Performance Section</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>N. W. Shock</td> <td>Scientist Emeritus</td> <td>NIA</td> </tr> </table> OTHER: Other workers who are associated with the Longitudinal Study describe their involvement in their individual reports.			PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA		A. H. Norris	Chief, Human Performance Section	CPB NIA		N. W. Shock	Scientist Emeritus	NIA
PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA											
	A. H. Norris	Chief, Human Performance Section	CPB NIA											
	N. W. Shock	Scientist Emeritus	NIA											
COOPERATING UNITS (if any)  Baltimore City Hospitals														
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch SECTION Human Performance Section														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
TOTAL MANYEARS: <div style="text-align: center;">9.25</div>	PROFESSIONAL: <div style="text-align: center;">2.45</div>	OTHER: <div style="text-align: center;">6.80</div>												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The Baltimore <u>Longitudinal Study</u> serves as a resource for scientists working in the field of <u>Gerontology</u> . It provides a well-described group of men and women between 20 and 96 years of age for studies of the <u>mechanisms of human aging</u> . Projects in <u>physiology</u> , <u>biochemistry</u> , <u>psychology</u> , <u>nutrition</u> , <u>pharmacology</u> , <u>endocrinology</u> , <u>sociology</u> , and <u>genetics</u> , have been carried out or are in progress.														

GRC/CPB-67

## Project Description:

Z01 AG 00015-20 CPB

Objectives: The Baltimore Longitudinal Study provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male and female volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 86% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 83% described themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% rated their health as good or excellent on both first and fifth visits.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retrieval and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: By June 30, 1978 a total of 1104 men and 85 women have participated in the testing program on one or more visits to GRC. Since the inception of the study 173 subjects have died and another 287 have withdrawn from the program, leaving an active sample of 644 men. As of this date, 795 men have completed three or more visits for testing, 614 visited five times or more, 370 eight times or more, 202 ten times or more, and 90 twelve times or more. In all, these subjects account for a grand total of 6406 participant visits.

The first female subject was tested on January 4, 1978. Of the 85 women tested thus far, 47 were wives of men already taking part in the study. At present, we have 360 male and 309 female volunteers on our waiting list.

A comparison of recent male and female recruits to the Baltimore Longitudinal Study follows:



Nearly all the 310 men tested in the first year of the current 2 year cycle were long-term participants whose social characteristics have been described elsewhere. It is of interest to determine how the 85 women entering the program since January 1, 1978 compare with the 40 men who came for testing for their first or second visit after July 1, 1977. The age distribution of these men reflects our continuing need to increase sample size under the age of 45 and over the age of 70. Educationally, these newer recruits vary somewhat from the pre-existing sample, with 70% reporting at least a bachelors degree as compared to 76% of subjects in the previous sample. A more striking difference is found in regard to marital status, with 78% of these men being currently married in contrast to 90% of the previous sample. The latter difference may be accounted for, in part, by this particular age distribution, and in part by the relatively recent rise in male age at marriage and increased marital instability in the general population. The fact that 5% of these men are non-white is consistent with our earlier recruitment.

Seventy-two percent of the women taken into the program were aged 50 and older. There has, however, been a rather large increase in the number of younger women among recent volunteers and the age distribution of participants in the coming year will be "normalized".

Women already in the program compare favorably with women on the waiting list with respect to educational attainment. Moreover, whether or not those admitted for testing were married to men in the program is largely unrelated to level of education. Of the women in the program, 59% held at least a bachelors degree--a figure which falls short of the 69% obtained for recent male recruits, but which is not surprising for women who are largely over age 50. As noted, 47 of the women were wives of male participants; only 20 of the 38 other women (53%) were not currently married. Since mortality rates are related to marital status, we will continue to monitor this variable as it impacts on our recruitment of new participants. A tentative goal of 650 women has been set.

#### Significance to Bio-Medical Research and the Program of the Institute:

A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age of onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Publications: None

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Age Changes in Human Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. H. Norris Chief, Human Performance Section CPB NIA  
S. P. Tzankoff Sr. Staff Fellow CPB NIA

OTHER: N. W. Shock Scientist Emeritus NIA

COOPERATING UNITS (if any)

A.T. Welford, Dept. of Psychology, University of Adelaide, South Australia  
G. Borkan, S.M. Garn, Ctr. for Human Growth & Development, U. of Michigan,  
Ann Arbor, Michigan

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

3.40

PROFESSIONAL:

.85

OTHER:

2.55

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms and the limitations of a variety of physical activities in old and young individuals. Muscular activity ranges from brisk walking on an inclined treadmill to tapping between targets of various widths drawn on paper and separated by various distances. Exercise responses are measured for blood pressure, heart rate, pulmonary ventilation, carbon dioxide elimination, and oxygen uptake. The oxygen cost of exercise is measured and compared to the total amount of physical work performed to estimate the mechanical efficiency of the subjects' neuromuscular and psychomotor control systems. Responses of the pulmonary system are interpreted in terms of standard spirometry and dead space (residual volume) measurements as well as studies of respiratory control. Limitations on performance imposed by cerebrovascular, cardiovascular, and pulmonary disease is assessed. Reflex time, reaction time and speed and accuracy of movement are measured and compared with exercise responses.

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake,  $\text{CO}_2$  elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of the stimulation of low oxygen and high oxygen and carbon dioxide in the inspired air are evaluated by pressure changes induced by occlusion of airflow ( $P_{0.1}$ ).

Subjects of the BLS continue to be evaluated for participating in the multi-purpose maximal treadmill exercise tests. Subjects are instructed to walk on the motor-driven treadmill at a constant speed of 5.6 km/hr. They start on the level and the grade is elevated by 3% increments every two minutes until either exhaustion or, at the discretion of the attending physician, the test is terminated. Before the walk, as well as during the exercise and recovery from it, electrocardiographic tracings are displayed and monitored on a CRT, recorded on magnetic tape, and periodic samples reproduced on strip-chart paper. In addition while walking, subjects breathe through a mouthpiece-valve arrangement which allows for the inspiration of room air and expiration into spirometers for measurement of pulmonary ventilation, and after gas analyses, for the calculation of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio for each level of exercise.

In the recovery phase venous blood samples are obtained at 3, 5, and 7 min. for the determination of lactic acid concentration, a by-product of anaerobic metabolism. Each subject who progresses through the test until exhaustion is asked to identify his limiting symptom, e.g., muscle pain, shortness of breath, general fatigue, and this response recorded. In addition, the investigator makes a subjective evaluation regarding whether or not the



performance represented a maximal effort. Exercise and recovery electrocardiographic tracings are evaluated for signs of ischemic coronary heart disease (ICHD) according to the World Health Organization standards.

Major Findings: PHYSIOLOGICAL VARIABLES AS INDICATORS OF BIOLOGICAL AGE.

In an analysis of data from the Baltimore Longitudinal Study of Aging (BLSA) physiological variables were adjusted to reflect biological age. The analysis was based on first visit cross-sectional data for 1086 men who were from 17 to 102 years of age. When physiological variables were related to life style variables, those physiological variables which required active cooperation of the subjects were more strongly related to life style variables than those which required only acquiescence on the part of the subjects. The 24 physiological variables included such things as strength, reaction times, tapping speed, pulmonary spirometry, skinfold thickness, heart rate and blood pressure. Nineteen life style variables included such things as education, marital status, caloric intake, smoking, and mortality status.

The 24 physiological variables were selected in two stages from a large number of tests in the BLSA. In the first stage, thirty-two variables were selected as representative of the organ systems being monitored in the BLSA and because they could be easily and accurately measured. In the second stage, screening procedures were used to identify variables which were most clearly age-related. Factor analysis revealed that these variables should not be combined but should be considered one at a time.

Biological age was calculated for each of the 24 variables by correcting for the effect of age. Residuals from piece-wise regression lines were used for this purpose. The data were then standardized by use of the Z-transformation. This resulted in a series of scores for each subject which reflected his physical status relative to his chronological age peers (i.e., reflected his biological age). Because the results were standardized it became possible to compare one variable with another as well as to compare the biological age of subjects with different chronological ages. The technique of pattern profile analysis was adopted to display graphically the biological age scores for the 24 variables.

The biological age profile was then used to investigate the sources of variation in biological age between individuals. The main analysis in this section utilized a set of "life style" variables to compare sub-populations in terms of mean biological age profiles. This analysis revealed that individuals in poor physical or mental health, or who were fatter, or less active, tended to be biologically older. It revealed that individuals who appeared older than their age are biologically older as well. It showed that participants in the study who have since died were biologically older at the time they were studied.

The 24 physiological variables in the biological age profile were ranked according to the strength of their overall association with the 19 life style variables. To produce the rankings, the difference between the two subgroup means within each physiological variable was computed. Thus,



vital capacity differences were computed for smokers and non-smokers, married and unmarried, etc. Then, for each physiological variable the mean of the standardized score value for these differences across all 19 life style variables was the score used for ranking the physiological variables.

Almost all of the highly ranked variables are derived from tests in which the subject must actively perform a task, rather than be passively measured. Such parameters include the three lung function variables (vital capacity, forced expiratory volume, and maximum breathing capacity) which rank first, fifth, and seventh. Tapping measures rank second and sixth, and the reaction times somewhat lower (tenth, eleventh, and thirteenth). The two strength related variables are also highly ranked with maximum work rate being third, and grip strength being ninth.

These results clearly suggest that aspects of aging which relate to performance of tasks are the most highly related to differences in life style. Certain other variables in the profile require the conscious cooperation of the subject to perform, but did not involve physical effort. All of these variables are characterized by moderate to low levels of association with life style. These include the test of visual memory (twenty-second), depth perception (twelfth), visual acuity (fifteenth), and auditory threshold (eighteenth).

Among the remaining variables, the low ranks of systolic and diastolic blood pressure (fourteenth and twentieth respectively) are suprising, because these variables are often viewed as important age indices. Creatinine clearance ranks seventeenth, and suggests that kidney function is not highly associated with the life style parameters. The three body composition measures have rather low rankings with basal metabolic rate sixteenth, creatinine excretion twenty-first, and cortical bone percent twenty-third. Two other blood protein measures are low in rank; serum albumin being nineteenth and hemoglobin twenty-fourth.

It appears from the summary results reported here that the biological age profile variables with the greatest overall association with life style are those involving active performance of physical tasks. This association may be due to the physiological complexity of these tasks, which makes them more susceptible to outside influence. For example, the test of tapping rate involves the interaction of the following systems: ability to hear instructions, ability to understand them, nervous system speed, arm mobility, muscular strength, and coordination. If several of these components have minor levels of impairment, the net effect on tapping rate may be substantial. It is, therefore, not suprising that life style should have its greatest effect on aspects of aging which require the coordination of a number of physiological systems.

WOMEN'S MAXIMAL TREADMILL EXERCISE RESPONSES. The addition of the women's longitudinal program in January 1978 has provided the opportunity of evaluating their exercise-induced physiologic responses. Aside from reducing the speed of the walk from the 5.6 km/hr used for men to 4.8 km/hr for

women, the test is not different from that of the men. Preliminary data evaluation on 58 women who have undergone the test shows that, like the men, women exhibit age-related decrements in capacity to perform muscular exercise. For example, when expressed in units of energy output, those women averaging 65 years of age perform only about 76% as well as women averaging 35 years of age. Compared with men of the same age, women's maximal capacities are about 30-40% lower. Maximal heart rates of women, measured at peak exercise, show the expected age-related decrements which are not significantly different from that of the men's. The present data on women compare well with the extremely limited literature data available.

Significance to Bio-Medical Research and the Program of the Institute:

The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants. Measurement of respiratory drive in relation to various stimuli will be evaluated in these participants.

Publications:

Borkan, G.A.: The Assessment of Biological Age During Adulthood. Doctoral dissertation. The University of Michigan, Ann Arbor, Michigan, 1978.

Garfinkel, F. and Fitzgerald: The effect of hyperoxia, hypoxia and hypercapnia on FRC and occlusion pressure in human subjects. Respiration Physiology, 1978, 33, 241-250.

MEDICIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00017-20 CPB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Age Relationships of Body Composition, Nutrition and Physical Activity		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	A.H. Norris      Chief, Human Performance Section R. Andres        Chief, Clinical Physiology Branch S.P. Tzankoff     Sr. Staff Fellow	CPB NIA CPB NIA CPB NIA
OTHER:	N.W. Shock        Scientist Emeritus J.D. Tobin         Medical Officer V. Elahi           Epidemiologist D. Elahi           Staff Fellow R. Aamodt          Chief, Whole Body Counter Section	NIA CPB NIA CPB NIA CPB NIA NM CC
COOPERATING UNITS (if any) P.T. Davis, Dept. of Medicine, University of Buffalo, Buffalo, N.Y. G. Borkan, S.M. Garn, Ctr. for Human Growth & Development, University of Michigan, Ann Arbor, Michigan		
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch		
SECTION Human Performance Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore Maryland 21224		
TOTAL MANYLARG:      2.50	PROFESSIONAL:        1.05	OTHER:                1.45
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  This study of the interrelationships of <u>body composition</u> , <u>nutrition</u> and <u>physical activity</u> is a <u>longitudinal study of aging</u> . It provides a description of these characteristics for participants in the <u>Baltimore Longitudinal Study</u> . It provides opportunity to relate changes in these basic characteristics of the individual participants to changes in other biochemical, physiological and psychological measurements. A variety of non-invasive techniques are employed. They include the <u>Behnke Anthropometric Index</u> , <u>skinfold thickness measurements</u> , <u>height</u> , <u>weight</u> , <u>twenty-four hour creatinine excretion</u> , <u>total body potassium determination</u> , <u>basal metabolism determinations</u> , <u>Garn X-ray fat thickness measurements</u> , a <u>diet diary</u> , and an <u>activity questionnaire</u> . Previously, measures of <u>total body density</u> and <u>total body water</u> have been made in longitudinal studies participants. <u>Body density corrected for differences in body water content</u> have been compared with the Behnke Index and other conventional <u>anthropometric indices</u> (such as <u>ponderal index</u> ).		

GRC/CPB-75



## Project Description:

Z01 AG 00017-20 CPB

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Analysis of the nutrition data followed the Schaie-Baltes model in order to separate the effect of aging from secular and cohort effects. The analytic objective was to determine how diet varies with age and how diet may have changed since the early 1960's. To answer these questions it is necessary to follow a group of men as they age to distinguish aging, secular and cohort effects on the nutritional variables of interest.

Food intakes from seven-day dietary records provided by 845 male participants were converted into nutrient intakes by trained dietitians and a computer program based DOA Handbook 8 as updated for these purposes. The subjects selected for inclusion in this study met the following criteria. First, each subject had to have supplied at least three dietary records. It was felt that a minimum of three observations was necessary to validly show a subject's trend in diet over time. Four hundred eighty-nine (489) subjects satisfied this criterion. Secondly, the subject had to have a record in each of three time periods, 1961-65, 1966-70 and 1971-75 (called Epoch 1, Epoch 2, and Epoch 3, respectively). These 5-year periods represent equal divisions of the time span in which dietary diaries were completed, that is 1961-75. This criterion was intended to maximize the length of the study period and to insure that all subjects entered observation at the same time. Two hundred fifty-six (256) subjects met this criterion. Finally, one had to be able to select a record from each time period such that the three records were in three consecutive five-year age categories. This allowed us to group the subjects into age quintiles and to follow each initial age group as a cohort in the analysis. One hundred ninety-nine (199) men satisfied all three of these criteria, and they ranged in age from 25 to 82 at the time of their first record. In 9 of the quintiles (ages 35-80) there were at least 5 subjects per quintile and analyses are based on those 9 cohorts.



Major Findings: Within each epoch and in the majority of cohorts, total caloric intake and absolute intake of fat and CHO decreased with increasing age. This is in accord with other researchers who have observed that caloric requirements decline with age due to a decrease in the basal metabolic rate and a reduction in physical activity. Protein intake tended to be constant over most of the age range studies, but the very old consumed less protein than the very young. Protein, fat and CHO intakes in Epoch 1 were greater than in 2 or 3.

The percent of total calories derived from fat, protein, and CHO did not change secularly over the 3 epochs. However, as the cohorts aged, there was a decrease in % calories from fat and an increase in % calories from CHO, while % calories from protein did not change. This is of interest with respect to the lower glucose tolerance of the older subjects; this defect obviously cannot be explained by a lower relative intake of CHO.

The mean intake of saturated fatty acids decreased both with increasing age and secularly. Intake of polyunsaturated fatty acids did not exhibit a consistent aging effect, but intake increased secularly. Since saturated fatty acid intake declined with age while polyunsaturated intake remained quite constant, the polyunsaturated/saturated fatty acid ratio tended to increase with increasing age. But the secular effect was more striking. The age-specific ratios increased over the 15 year study period reflecting the secular decrease in saturated fatty acid intake and the secular increase in polyunsaturated fatty acid intake. The mean value for the ratio of polyunsaturated to saturated fatty acids increased from .34 in Epoch 1 to .43 in Epoch 3, a 26% increase.

Cholesterol intake did not show an aging effect but the vertical separation of the profiles indicated a strong secular effect. The mean cholesterol intake in the 9 age quintiles declined from 578 mg in Epoch 1 to 462 in Epoch 3, a decrease of 20%.

Over this study period of 1961 to 1975, there was a secular decrease in the absolute intake of all the nutrients considered here with one exception--intake of polyunsaturated fatty acids increased. However, relative caloric intake of fat, protein, and carbohydrates did not change. The secular increase in the polyunsaturated to saturated fatty acid ratio and the secular decrease in cholesterol intake were particularly striking and consistent across all ages and all cohorts. These latter changes may be a result of public health efforts to alter the American diet.

#### Significance to Bio-Medical Research and the Program of the Institute:

Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological age per se may, therefore, be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition, food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

#### Publications:

Borkan, G.A. and Norris, A.H.: Fat redistribution and the changing body dimensions of the adult male. Human Biology, 49:495-514, 1977.

Posner, D.B., Russell, R.M., Absood, S., Connor, T.B., Davis, C., Martin, L., Williams, J.B., Norris, A.H., and Merchant, C.: Effective 25-hydroxylation of vitamin D<sub>2</sub> in alcoholic cirrhosis. Gastroenterology 74:866-870, 1978.

Tzankoff, S. P. and Norris, A. H.: The effect of muscle mass decrease on age-related BMR changes. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 43:1001-106, 1977.

Tzankoff, S.P. and Norris, A.H.: Longitudinal changes in basal metabolism in man. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. (in press).

## PERIOD COVERED

October 1, 1977 to September 30, 1978

## TITLE OF PROJECT (80 characters or less)

Marital, Sexual and Social Factors in Aging

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Martin	Sociologist	CPB NIA
OTHER:	S.M. Harman	Medical Officer	CPB NIA
	L. Giambra	Psychologist	LBS NIA

## COOPERATING UNITS (if any)

Baltimore City Hospitals  
H. Seideman, University of Maryland Baltimore Campus

## LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

## SECTION

Human Performance Section

## INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

## TOTAL MANYEARS:

.80

## PROFESSIONAL:

.60

## OTHER:

.20

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER☐ (a1) MINORS ☒ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Systematic data concerning sexual, marital and social experience were obtained by structured interviews with males taking part in the Baltimore Longitudinal Study of Aging. In analysis 188 subjects aged 60-79 and married at interview were divided into thirds according to quantity of sexual activity reported for the preceding year. Subjective and behavioral variables were then related to subjects classified as least active, moderately active and most active sexually in order to identify factors affecting level of sexual functioning at these ages. Major correlates of being sexually most active were: a strong commitment to religious values, early farm residence, high socio-economic status, more abundant sexual activity during the earlier years of life, continued erotic responsiveness to visual stimuli, and unimpaired potency. Future plans are to relate variables derived from these interviews to such diagnostic entities as coronary artery disease, hyperlipidemia and other disabilities.

GRC/CPB-79



## Project Description:

Z01 AG 00018-12 CPB

Objectives: Current objectives are to: 1) identify factors or mechanisms responsible for the variation in frequencies of sexual activity reported by older respondents, 2) learn whether coronary artery disease or other diseases of unknown etiology may be related to some aspect of marital or sexual experience, and 3) interview male members of the longitudinal study who have not yet completed an interview.

Methods Employed: The vast majority of longitudinal study members have now been interviewed with respect to their history of marriage and sexual activity. In requesting these interviews, the investigator described study objectives in detail, provided assurance of confidence, and emphasized the voluntary nature of such a contribution. Over time the refusal rate has remained at 2 to 3 percent.

To aid communication and to insure systematic data collection, all questions asked by the investigator had been memorized along with whatever categories were required to classify responses. To help with establishing rapport and to generate data not elsewhere obtained, aspects of residential, occupational, educational, religious, military and parental-home experience were reviewed before introducing questions about marital adjustment or sexual conduct. The data obtained are in many respects unique and are believed to be of unusual quality because of the high socio-economic status of respondents and their evident interest in this part of the study.

Major Findings: Over the year, the remaining volunteers participating in Dr. S. M. Harman's study of endocrine factors in relation to sexual functioning were interviewed and the data obtained made available for his analysis (Identification Z01 AG 00023 CPB).

In last year's report, an analysis was described in which 188 married respondents aged 60-79 at report were subdivided in three nearly equal groups according to the amount of sexual activity reported for the preceding year. These groups were then related to numerous variables derived from the interview in an effort to identify significant correlates of sexual functioning. From the results obtained it was apparent that a large share of the variation in sexual frequencies observed was attributable to individual differences in sexual vigor that had persisted over much of the earlier life history. No significant part of this variation appeared to be the product of different sexual attitudes, differences in marital adjustment or impaired potency due to negative affect or performance anxiety, as has often been assumed. A large share of research time this past year went into preparing these data for publication.

Various attempts were made during the year to initiate a search for additional correlates of male sexual functioning among the many physiologic and psychologic data obtained by other members of the research staff. This effort was prompted by: 1) the finding that differences in male sexual functioning were unrelated to emotional factors in the life history, 2) evidence indicating that the factors responsible for this variation are not necessarily age-dependent, and 3) still other evidence which strongly suggests that many older



men become oblivious of sexual needs because of an increasing inability to translate available visual stimuli into erotic arousal. According to this interpretation, the maintenance of sexual desire in old age depends upon the integrity of that part of the brain which is most involved in the translation of visual and other imaginal information into erotic impulses.

Except for the observation that certain threshold values of circulating testosterone are essential for male sexual function, research has yet to identify any specific factor which could account for individual differences in sexual vigor. Thus, the hypothesis that altered cognitive functioning is implicated by retrospective evidence deserves to be pursued. It is for this reason that the present investigator has joined with L. Giambra and H. Seideman, psychologist, in planning the kinds of analyses that should be done. The effort has been slowed somewhat by the difficulty of explaining to other staff members the need for a relatively unbiased review of factors in the search for correlates of sexual functioning, in lieu of limiting the search to those few factors for which a rationale can presently be provided.

Significance to Bio-Medical Research and the Program of the Institute:

Because of the probabilities involved, fishing expeditions for significant correlates are not easily defended. Nevertheless, it appears to be a logical course to follow when a search can be inexpensively done, the circumstances allowing such investigation are not likely to be duplicated again for a long while, and so little is known about the mechanisms involved in male sexual functioning.

Proposed Course: In addition to pursuing the above analyses, it is planned to compare longitudinal subjects diagnosed as having coronary artery disease and matched controls in an effort to discern differences in marital, sexual or social attributes. This analysis is expected to be the first of a similar series of studies involving: diabetes, hypertension, hyperlipidemia, and other disease entities.

Publications:

Giambra, L.M. and Martin, C.E.: Sexual daydreams and quantitative aspects of sexual activity: some relations for males across adulthood. Archives of Sexual Behavior, 6:497-505, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00021-15 CBP												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Dermatoglyphics in: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">1. Populations</td> <td style="width: 33%;">4. Families</td> <td style="width: 33%;"></td> </tr> <tr> <td>2. Medicine</td> <td>5. Twins</td> <td></td> </tr> <tr> <td>3. Aging</td> <td>6. Methodology</td> <td></td> </tr> </table>			1. Populations	4. Families		2. Medicine	5. Twins		3. Aging	6. Methodology				
1. Populations	4. Families													
2. Medicine	5. Twins													
3. Aging	6. Methodology													
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: C.C. Plato</td> <td style="width: 33%;">Geneticist</td> <td style="width: 33%;">CPB NIA</td> </tr> <tr> <td>OTHER: D.C. Gajdusek</td> <td>Chief, Lab. of Central Nervous System Studies</td> <td>CNS NINCDS</td> </tr> <tr> <td>R. Garruto</td> <td>Sr. Staff Associate</td> <td>CNS NINCDS</td> </tr> <tr> <td>B.D. Bricker</td> <td>Computer Specialist</td> <td>CPB NIA</td> </tr> </table>			PI: C.C. Plato	Geneticist	CPB NIA	OTHER: D.C. Gajdusek	Chief, Lab. of Central Nervous System Studies	CNS NINCDS	R. Garruto	Sr. Staff Associate	CNS NINCDS	B.D. Bricker	Computer Specialist	CPB NIA
PI: C.C. Plato	Geneticist	CPB NIA												
OTHER: D.C. Gajdusek	Chief, Lab. of Central Nervous System Studies	CNS NINCDS												
R. Garruto	Sr. Staff Associate	CNS NINCDS												
B.D. Bricker	Computer Specialist	CPB NIA												
COOPERATING UNITS (if any)  See attached page.														
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch														
SECTION Human Performance Section														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
TOTAL MANYEARS: .60	PROFESSIONAL: .40	OTHER: .20												
CHECK APPROPRIATE BOX(ES)														
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER														
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)														
<p>           This project represents an ongoing joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the collection, evaluation and interpretation of <u>dermatoglyphic data</u>. Specifically the objectives of this project are: 1) to study the <u>distribution of dermatoglyphics among the various human populations (population dermatoglyphics)</u>; 2) to establish the dermatoglyphic frequencies in normal control samples (<u>control dermatoglyphics</u>); 3) to establish dermatoglyphic markers in various diseases (<u>clinical dermatoglyphics</u>); 4) to study the <u>dermatoglyphics of the aged</u>; 5) to study the <u>genetics of dermatoglyphics</u>; and, 6) to utilize dermatoglyphics as an added tool in twin diagnoses (<u>twin dermatoglyphics</u>).         </p>														

GRC/CPB-82

Cooperating Units:

Z01 AG 00021-15 CPB

1. W. Wertelecki  
Department of Genetics  
University of South Alabama  
Mobile, Alabama
2. J. T. Schwartz  
Division of Hospitals & Clinics  
Bureau of Medical Services  
Health Service Administration  
USPHS, West Hyattsville, Md.
3. R. MacLennan  
International Agency for Research on Cancer  
WHO, Lyon, France
4. M. Alpers  
Institute of Medical Research  
Goroka, New Guinea
5. C. Bartsocas  
Department of Pediatrics  
University of Athens  
Athens, Greece
6. A. D'Alessandro  
International Center for Medical Research  
Universidad Del Valle  
Calik, Colombia
7. M. T. Newman  
Department of Anthropology  
University of Washington  
Seattle, Washington
8. R. W. Hornabrook  
New Guinea Institute of Medical Research  
Wadestown, Wellington, New Zealand
9. Y. Ahuja  
Department of Genetics  
Osmania University  
Hyderabad, India
10. G. M. Flickinger  
Department of Biology  
Xavier University of Louisiana  
New Orleans, Louisiana

11. Paul T. Paker  
Chairman, Department of Anthropology  
Pennsylvania State University  
College Station, Pennsylvania
12. W. Pollitzer  
Department of Anatomy  
The University of North Carolina at Chapel Hill  
Chapel Hill, North Carolina
13. J. Larrick  
Duke Medical School  
Duke University  
Durham, North Carolina
14. B. Schaumann  
Neurology Section  
The Veterans Administration Hospital and  
The University of Minnesota  
Minneapolis, Minnesota
15. R. G. Schamschula  
The Institute of Dental Research  
The United Dental Hospital of Sydney  
Sydney, Australia

Project Description:

Objectives: This ongoing project represents an extensive collaborative effort, in conjunction with WHO and other national and international institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: (1) to establish the distribution of dermatoglyphic features in various populations, with special emphasis on primitive and other isolated groups in the South Pacific and other parts of the world. (2) To establish associations between dermatoglyphic features and specific clinical anomalies. (3) To study the dermatoglyphic frequencies in different age groups. (4) To investigate the genetic aspects of dermatoglyphics through family and twin data. (5) To improve and standardize the international methodology and nomenclature on dermatoglyphics.

The overall purpose of these studies is to utilize the dermatoglyphic markers in an effort to study the genetic structure of different populations, and to provide additional tools for studying the etiology of certain diseases.

Methods: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results. We developed new methods and computer programs for studying and analyzing the dermatoglyphic data. These methods have been accepted and are utilized by other laboratories here and abroad.



Major Findings: 1) Dermatoglyphics and Aging: Dermatoglyphic comparisons between 7 year old children and adults from the Baltimore Longitudinal Study and a second adult sample from South Carolina indicated that there is a decreasing frequency of palmar patterns and an increase in the transversality of the palmar ridges. There was also a significant increase of aberrant simian palmar flexion creases among the adults.

Population Studies: Completed an exhaustive review of the dermatoglyphics of the Amerindians. We also completed the evaluation of the dermatoglyphics of 75 isolates from Australasia. The latter represents most of the dermatoglyphic studies carried out in that region. The results of the above studies will be considered along with the other genetic markers studies in these two regions.

Twin Dermatoglyphics: These studies indicated that even though dermatoglyphic traits are genetically controlled, their final phenotype is influenced to a considerable extent by intrauterine factors.

Clinical Dermatoglyphics: 1) There were no significant differences between the dermatoglyphics of patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam and non-affected Guamanian (Chamorro) controls. 2) The dermatoglyphics of the Chamorros are significantly different from those of the other Micronesians. These results agree with those we reported earlier on the blood group investigations.

Significance to Bio-Medical Research and the Program of the Institute:

1) To utilize dermatoglyphics as an added genetic marker in the overall study of aging. 2) To provide internationally standardized dermatoglyphic frequencies which could be used as control data in the studies of disease associations and for facilitating factor analyses in evaluating the studies of genetic relationship among isolates.

Proposed Course: To continue this project by further evaluating the data at hand and by the collection of additional clinical and population dermatoglyphic data from subjects of all ages.

Publications:

Plato, C.C.: Dermatoglyphics and aging. J. Geront. 11:31-38, 1978.

Plato, C.C., Gajdusek, D.C. and MacLennan, R.: The Dermatoglyphics of the Peoples of New Guinea: A review. In J. Mavalwala (Ed.): Dermatoglyphics and International Perspective. The Hague, Mouton Press, 1978, pp. 195-214.

Plato, C.C., Schwartz, J.T. and Wertelecki, W.: Dermatoglyphic investigations in twins and siblings. Acta Geneticae Medicae et Gemellologiae (Roma) 25:167-173, 1976.

Plato, C.C., Wertelecki, W. and Schwartz, J.T.: Normal and aberrant palmar creases in twins and siblings. Acta Geneticae Medicae et Gemellologiae (Roma) 25:174-176, 1976.

Schaumann, B., Plato, C.C. and Wertelecki, W.: Selection of appropriate controls in dermatoglyphic studies. Am. J. Physical Anthropol. 45:434, 1978.

Plato, C.C.: Dermatoglyphics and their significance in aging studies. Digest, Geriatrics. In press.

Pollitzer, W. and Plato, C.C.: Anthropology and Dermatoglyphics. In Wertelecki, W., Plato, C.C. and Bergsma, D. (Eds.): Dermatoglyphics--50 Years Later. The American Dermatoglyphics Association and The National Foundation March of Dimes. New York, Alan Liss Publishers. In press.

Garruto, R.M., Plato, C.C., Hoff, C.J., Newman, M.T., Gajdusek, D.C. and Baker, P.T.: Characterization and Distribution of Dermatoglyphic Features in Eskimo and North, Central and South American Indian Populations. In Wertelecki, W., Plato, C.C. and Bergsma, D. (Eds.): Dermatoglyphics--50 Years Later. The American Dermatoglyphics Association and The National Foundation March of Dimes. New York, Alan Liss Publishers. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER  Z01 AG 00022-02 CPB	
PERIOD COVERED October 1, 1977 to September 30, 1978					
TITLE OF PROJECT (80 characters or less)  Investigations of Osteoarthritis and Bone Loss					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:		C.C. Plato		Geneticist	
		A.H. Norris		Chief, Human Performance Section	
				CPB NIA	
				CPB NIA	
OTHER:		D.C. Gajdusek		Chief, Lab. of Central Nervous	
				Systems Studies	
		R.M. Garruto		Sr. Staff Associate	
				CNS NINCDS	
				CNS NINCDS	
COOPERATING UNITS (if any) Tecumseh Michigan Community Health Study The University of Michigan, Ann Arbor, Michigan					
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch					
SECTION Human Performance Section					
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224					
TOTAL MANYEARS:		PROFESSIONAL:		OTHER:	
.70		.50		.20	
CHECK APPROPRIATE BOX(ES)					
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS		<input type="checkbox"/> (b) HUMAN TISSUES		<input type="checkbox"/> (c) NEITHER	
<input type="checkbox"/> (a1) MINORS		<input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)					
<p><u>Osteoarthritis</u> and <u>bone loss</u> are the two principal age related changes of the human skeleton. Even though these changes are considered inherent to aging, they may result in incapacitating ailments. The advanced cases of osteoarthritis (degenerative joint disease) produce severe restriction of movement associated with pain. Advanced bone loss may result in <u>osteoporosis</u> and frequent <u>bone fractures</u>. Most prominent are vertebral compression fractures and fractures of the femoral neck.</p> <p>This project deals with the epidemiological, genetic and longitudinal aspects of osteoarthritis and bone loss among (1) the participants of the Baltimore Longitudinal Study, (2) in a sample of normal Guamanians (Chamorros), and (3) among patients afflicted with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam.</p>					
GRC/CPB-87					

Objectives: The objectives of this project are: 1) To study the epidemiological, genetic and longitudinal aspects of both osteoarthritis and bone loss. 2) To ascertain possible associations between these two types of bone changes and other diseases, as well as several selected physiological and anthropometric variables. 3) To study the prevalence of osteoarthritis and osteoporosis in the thoracic spine as well as other parts of the skeleton. 4) To ascertain the lateral functional dominance among the Baltimore Longitudinal Study participants and the possible relationship between functional dominance (handedness) and the diseases under investigation. 5) To compare the degree of bone loss and osteoarthritis between the Guamanians suffering from ALS/PD and a sample of non-affected Chamorro (Guamanian) controls.

Methods Employed: Radiographs were graded for osteoarthritis for each of the proximal and distal interphalangeal joints utilizing the internationally accepted grading system of J. H. Kellgren. Grades 0 and 1 were considered negative (normal), and grades 2, 3 and 4 were considered as having osteoarthritis (affected). Bone measurements were made for the total width, medullary width and the length of the second metacarpal bone. From these measurements we calculated the combined cortical thickness, the cortical area, the cortical area index and the cortical volume. The cross-sectional study was based on both the left and the right hands. The longitudinal study included participants with at least three x-rays; only left hands analyzed. Lateral functional dominance was established through a series of tests involving gross as well as fine manipulations of the hands. Foot and eye preferences were also ascertained as well as grip strength. In addition to the participants of the Baltimore Longitudinal Study and the Guamanian ALS/PD patients and non-affected controls, we also studied the bilateral aspects of osteoarthritis in 100 male and 100 female participants, 55 years of age and older, of the Tecumseh Michigan Community Study.

Major Findings: Osteoarthritis was found to be more prevalent in the distal than the proximal interphalangeal joints of the hand. The right hands are more frequently and more severely affected with osteoarthritis than the left. Regardless of hand or type of joint, the fifth digits (little fingers) are the most vulnerable to this disease. The occurrence of osteoarthritis in the distal interphalangeal joints is longitudinally related to age, while its presence in the proximal joints is not. These results suggest that the occurrence of osteoarthritis in the distal and proximal interphalangeal joints may be of different etiology.

Our cross-sectional studies confirmed previous findings that bone loss is a universal phenomenon associated with aging. Through our longitudinal study we also showed, for the first time, that this phenomenon also holds true on the individual level. The second metacarpal bones of the right hands have more bone than those of the left hands regardless of lateral dominance, when handedness was ascertained through bilateral differences in grip strength; however, the left hands of the "left handed" participants as a rule had higher bone content than the left hands



of the "right handed". These results suggest that, due to inherent factors, the right hands have higher bone content than the left. This bilateral difference is, however, reduced in the left handed due to increased activity of their left hands. The preliminary results on the comparison between ALS Chamorro patients and non-affected Chamorro controls indicated that there is a decrease in bone content among the former. This reduction of bone is not so evident in the patients with Parkinsonism Dementia. Whether the excess bone loss seen in the ALS patients is solely due to inactivity brought about by the paralysis or whether part of this loss preceded the bed-ridden stage of the disease cannot be ascertained from the present data. We are investigating these possibilities by collecting x-rays of the hands of ALS suspects or patients who are still at the very early stage of the disease.

Significance to Bio-Medical Research and the Program of the Institute:

Loss of bone tissue and deterioration of the joints are significant causes of disability and death in older people. Determination of the existence and degree of disease in relatively well ambulatory people provides a unique opportunity to compare bone loss and degeneration with other characteristics of these people. Nutrient intakes, activity levels and muscle strength among other things may be compared.

Proposed Course: To finish the evaluation of the collected data and publish the results. To continue the collection of bilateral x-rays from the male as well as from the female participants of the Baltimore Longitudinal Study. Now that the prevalence and rates of change in bone loss and joint degeneration have been described, comparisons of these processes with other characteristics and processes in these participants will be undertaken. Such things as nutrient intakes, medications, physical activity and muscle strength will be considered. To expand the data on the ALS/PD patients, suspected patients and non-affected Guamanian controls.

Publications:

Plato, C.C. and Norris, A.H.: Osteoarthritis of the hand: Age specific joint-digit prevalence rates. J. Epidemiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00027-02 CPB										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less)  Functional Lateral Dominance and Bilateral Asymmetry												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">C.C. Plato</td> <td style="width: 33%;">Geneticist</td> <td style="width: 15%;"></td> <td style="width: 15%;">CPB NIA</td> </tr> <tr> <td>OTHER:</td> <td>A.H. Norris</td> <td>Chief, Human Performance Section</td> <td></td> <td>CPB NIA</td> </tr> </table>			PI:	C.C. Plato	Geneticist		CPB NIA	OTHER:	A.H. Norris	Chief, Human Performance Section		CPB NIA
PI:	C.C. Plato	Geneticist		CPB NIA								
OTHER:	A.H. Norris	Chief, Human Performance Section		CPB NIA								
COOPERATING UNITS (if any)												
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch												
SECTION Human Performance Section												
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224												
TOTAL MANYEARS: <div style="text-align: center;">0</div>	PROFESSIONAL: <div style="text-align: center;">0</div>	OTHER: <div style="text-align: center;">0</div>										
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this investigation is to evaluate the various forms of <u>lateral functional dominance</u> , such as <u>hand</u> , <u>eye</u> and <u>foot preference</u> and to study their association with normal or <u>abnormal bilateral asymmetry</u> (osteoarthritis and bone loss) as well as with <u>normal bilateral asymmetry</u> seen in dermatoglyphics.												
COMBINED INTO PROJECT NUMBER: Z01 AG 00022-02 CPB												

GRC/CPB-90

Project Description:

Z01 AG 00027-02 CPB

Combined into Project No. Z01 AG 00022-02 CPB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00028-02 CPB
PERIOD COVERED <b>October 1, 1977 to September 30, 1978</b>		
TITLE OF PROJECT (80 characters or less) <b>Epidemiological and Genetic Studies of Amyotrophic Lateral Sclerosis/          Parkinsonism Dementia (ALS/PD) Complex of Guam</b>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	C.C. Plato	Geneticist CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies CNS NINCDS
	R.M. Garruto	Sr. Staff Associate CNS NINCDS
COOPERATING UNITS (if any)  <b>C &amp; F Research Center, NINCDS</b>		
LAB/BRANCH <b>Gerontology Research Center, Clinical Physiology Branch</b>		
SECTION <b>Human Performance Section</b>		
INSTITUTE AND LOCATION <b>NIA, NIH, Baltimore, Maryland 21224</b>		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.30	.20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this study is to investigate the <u>genetic and epidemiological factors</u> contributing to the very high incidence of <u>Amyotrophic Lateral Sclerosis and Parkinsonism Dementia (ALS/PD)</u> on Guam. Also to evaluate the <u>distribution of the various established genetic and anthropological markers</u> among the normal Guamanian population and compare them with those of the ALS/PD patients.		

GRC/CPB-92



Objectives: Our involvement in the multidisciplinary project has three objectives: 1) to identify the epidemiological variables which contribute to the very high incidence of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia (ALS/PD) Complex of Guam; 2) to ascertain the possible changes in the incidence of ALS/PD; 3) to determine the extent of genetic involvement in the etiology of the disease; and 4) to study the distribution of several established genetic markers such as blood groups, serum proteins, red cell enzymes and dermatoglyphics in the normal Guamanian (Chamorro) population to be used as controls in comparisons with patients.

Methods: For the past twenty years efforts have been made to ascertain and to study all cases of ALD/PD Complex on Guam. Most pertinent epidemiological variables relating to the disease have been evaluated. Eighteen years ago we also initiated two genetically oriented studies. 1) The patient-control registry panels consistent of 136 patients and 136 individually matched controls and their respective sibs, parents, offspring and spouses. 2) The collection of extensive family pedigrees of the ALS/PD patients and a complete genealogy of all the individuals born in the village of Umatac, which has the highest concentration of ALS patients. These programs have been followed up, updated and expanded through the years by other investigators. We are presently organizing and evaluating the epidemiological and genetic data accumulated during the past twenty years. All death certificates from 1970 to date are also being scrutinized for ALS/PD as well as other causes of death.

Major Findings: There is evidence that the prevalence of ALS/PD on Guam has been decreasing during the past ten years. This decrease is mainly due to the reduction of the prevalence of ALS among the male. The blood group comparison between patients with the ALS only and non-affected Guamanian controls showed no significant differences, while patients with Parkinsonism Dementia (PD) only showed significant difference in the ABO system. Patients with both ALS and PD symptoms, however, have significantly different frequencies in the ABO, Rh, MNS, and the Diego systems than those of the controls. The significance of the blood group differences between the individual symptoms (ALS, PD, ALS+PD) and the controls and the lack of significant differences between all of them combined (ALS/PD Complex) and the controls is presently being evaluated. Dermatoglyphic comparisons between ALS/PD patients and random Guamanian controls showed no significant differences. The ALS patients have significantly lower bone measurements than non-affected controls of the same age.

Significance to Bio-Medical Research and the Program of the Institute:

The ultimate goal of this multidisciplinary program is not only to elucidate the etiology of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam, but also to provide a model for studying other neurological diseases and dementias which are for the most part diseases of old age.

Proposed Course: To reevaluate the last updating (February 1977) of the patient-control registries, pedigrees. Also to review all the epidemiological and genetic data accumulated during the past twenty years.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00093-06-CPB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cellular Basis of Regulation of the Humoral Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. A. Nordin  
Other: P. L. Mann

Research Chemist  
Visiting Fellow

CPB NIA  
CPB NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

2.6

PROFESSIONAL:

1.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulatory mechanisms of lymphoid cells are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immuno-senescence is being investigated.

GRC/CPB-94

## Project Description:

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

## Methods Employed:

(1) The in vitro culture techniques and the assay for plaque-forming cells are routine methods.

(2) The carbonyl-iron treatment of spleen cells is accomplished by adding 25 mg of sterile carbonyl iron to  $100 \times 10^6$  normal spleen cells. After a 30 minute incubation at  $37^\circ\text{C}$  in a 5%  $\text{CO}_2$  environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells are used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added. Supernatants of peritoneal cells collected 24 hours after culture initiation are used in some instances instead of the peritoneal cells.

(4) DAGG-Ficoll is prepared by modifying Ficoll by introducing carbonyl methyl amino-ethyl groups to which is added the tri-peptide glycine-glycyl-alanyl with the terminal alanine substituted with a single dinitro-phenol haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings: Spleen cells from individual old C57Bl/6J mice (20-26 months) cultured in vitro in the presence of a macrophage derived factor and mercaptoethanol show an increased antibody response to a T-independent antigen. However, the response remains significantly below that observed with spleen cells from young control mice indicating that either the size of the responding clone decreases with age or that the cells within the clone have a reduced proliferation potential. Preliminary experiments favor the possibility of reduced proliferative ability.

Spleen cells depleted of thymus-derived cells and cultured in vitro in the presence of the macrophage derived factor(s) show a certain level of antibody formation to a T-independent antigen. If normal thymus-derived spleen cells are added to cultures, the level of antibody-formation is dramatically increased. This finding strongly suggests that normal thymus-derived lymphocytes influence the response of bone marrow derived lymphocytes even to a thymus independent type antigen. The increased response is apparently due to any increase in the burst size of the antigen stimulated B-lymphocytes.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: The response of aging mice to the T-independent antigen, DAGG-Ficoll, will be re-examined in light of the evidence that normal thymus-derived lymphocytes markedly influences the in vitro immune response. The aim of these studies will be to accurately estimate both the size of this antigen reactive clone and the proliferation potential of the aging B-lymphocytes.

Attempts will be made to purify the factor(s) present in the macrophage culture supernates. The purified material will be used in efforts to establish the mechanism by which the factor(s) influence the activity of lymphocytes.

#### **Publications:**

Nordin, A. A. and Adler, W. H.: The effect of aging on in vitro cellular interactions. Fifth Annual Irwin Strassburger Memorial Seminar on Immunology. Developmental Immunology, in press.

Mann, P. L.: The effect of various dietary restricted regimes on some immunological parameters of mice. Growth 42: 87-103, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00094-05-CPB						
PERIOD COVERED <b>October 1, 1977 to September 30, 1978</b>								
TITLE OF PROJECT (80 characters or less)  <b>Characterization of Immune System of Aging Mice with Immunodeficiency</b>								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">A. A. Nordin</td> <td style="width: 33%;">Research Chemist</td> </tr> <tr> <td></td> <td></td> <td>CPB NIA</td> </tr> </table>			PI:	A. A. Nordin	Research Chemist			CPB NIA
PI:	A. A. Nordin	Research Chemist						
		CPB NIA						
COOPERATING UNITS (if any) None								
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch								
SECTION Clinical Immunology Section								
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224								
TOTAL MANYEARS: 1.0	PROFESSIONAL: .2	OTHER: .8						
CHECK APPROPRIATE BOX(ES) <table style="width: 100%;"> <tr> <td><input type="checkbox"/> (a) HUMAN SUBJECTS</td> <td><input type="checkbox"/> (b) HUMAN TISSUES</td> <td><input checked="" type="checkbox"/> (c) NEITHER</td> </tr> <tr> <td><input type="checkbox"/> (a1) MINDRS</td> <td><input type="checkbox"/> (a2) INTERVIEWS</td> <td></td> </tr> </table>			<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input checked="" type="checkbox"/> (c) NEITHER	<input type="checkbox"/> (a1) MINDRS	<input type="checkbox"/> (a2) INTERVIEWS	
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<input type="checkbox"/> (a1) MINDRS	<input type="checkbox"/> (a2) INTERVIEWS							
SUMMARY OF WORK (200 words or less - underline keywords)  <p>             The purpose of this project is to characterize the <u>in vitro cell-mediated immunity</u> in young and aging mice of various genetic strains. The relationship between the <u>immunodeficiency in aging</u> and the regulation, in the form of suppression of the immune system, is investigated. The topics of present interest are: (1) the regulatory mechanisms of the <u>in vitro</u> development of <u>cytotoxic lymphocytes by suppressor cells</u>, (2) the characterization of suppressor cells, and (3) the characterization of the immunodeficiency in aging and its relationship with suppressor cells.           </p>								

GRC/CPB-97

## Project Description:

Objectives: The goal of this project is to characterize the immune system in young and aging mice of various genetic strains. The relationship between the immunological disorders and the regulation, in the form of suppression of the immune system, is investigated.

Methods Employed: (1) A modification of the spleen cell culture system of Mishell and Dutton is used. Spleen cells from individual mice or pooled spleen cells are cultured with mitomycin-C treated or irradiated allogeneic spleen cells,  $F_1$  spleen cells or heterologous erythrocytes at  $37^{\circ}\text{C}$  for various days. The double chamber culture system is also used.

(2) Cytotoxicity assay -  $^{51}\text{Cr}$  labelled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

(3) Plaque-forming cell assay-routine technique used to detect IgM and IgG antibody-producing cells.

Major Findings: Spleen cells from mice 22-32 months of age were assayed individually for the in vitro development of cytotoxic lymphocytes as well as their capacity to elaborate suppressive factors. The degree of suppression expressed by the aging mouse spleen cells is inversely related to the level of cytotoxic lymphocytes expressed. The suppressive factor(s) functions by inhibiting the cellular proliferation of lymphoid cells.

Significance to Biomedical Research and the Program of the Institute: This proposal offers two main significant contributions: (1) the regulatory mechanism of cell-mediated immunity in young and aged mice, and (2) the mechanism of the immunosenescence of cell-mediated immunity.

Proposed Course: Attempts to isolate the factor(s) elaborated into the supernate of mixed lymphocyte cultures that suppresses the development of cytotoxic lymphocytes will be undertaken using affinity chromatography. A reliable method for quantitating the biological activity of the factor(s) will be established in an effort to determine if lymphoid cells from aging mice produce higher levels of the factor(s) than their young counterparts.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00095-05-CPB																	
PERIOD COVERED October 1, 1977 to September 30, 1978																			
TITLE OF PROJECT (80 characters or less) The Role of Cell Membrane Structures on Cellular Recognition																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																			
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">W. H. Adler</td> <td style="width: 40%;">Medical Officer, PHS</td> <td style="width: 20%;">CPB NIA</td> </tr> <tr> <td rowspan="4">Other:</td> <td>J. E. Nagel</td> <td>Clinical Associate, PHS</td> <td>CPB NIA</td> </tr> <tr> <td>K. H. Jones</td> <td>Staff Fellow</td> <td>CPB NIA DOD 7/15/78</td> </tr> <tr> <td>M. A. Brock</td> <td>Research Biologist</td> <td>CPB NIA</td> </tr> <tr> <td>R. K. Saxena</td> <td>Visiting Fellow</td> <td>CPB NIA EOD 7/1/78</td> </tr> </table>			PI:	W. H. Adler	Medical Officer, PHS	CPB NIA	Other:	J. E. Nagel	Clinical Associate, PHS	CPB NIA	K. H. Jones	Staff Fellow	CPB NIA DOD 7/15/78	M. A. Brock	Research Biologist	CPB NIA	R. K. Saxena	Visiting Fellow	CPB NIA EOD 7/1/78
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SECTION Clinical Immunology Section																			
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224																			
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SUMMARY OF WORK (200 words or less - underline keywords)  <u>Lymphoid cells</u> from mice exhibit rhythms of <u>mitogen responsiveness</u> and <u>viability in culture</u> . The nature of these circannual and lunar rhythms is unknown. The studies on <u>cytotoxic T cell memory</u> and mitogen responsiveness were slowed by a decrease in the young animals functional ability.																			

GRC/CPB-99

## Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable populations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to arrive at methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. The tests of immune functions will include responses to mitogen and antigen in in vitro culture conditions, responses to antigens and oncogenic stimuli in vivo, and the development of immunologically competent cells in in vitro environments. These studies will give a better understanding of age-related immunodeficiency.

Methods Employed: The basis of most functional assays will be in vitro culture systems. There will be both short term for the investigation of mitogen and antigen responsiveness and for the generation of antibody-forming cells, and longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic tumor cells. The cells will be from various lymphoid organs and from varying aged donors. The cells will be treated by physical separation methods and with specific antisera to eliminate certain populations, or to quantitate various cellular population representation.

Major Findings: Over the past 3 years data has been collected on the level of response of mouse spleen cells to T cell mitogen and the dose response profile to Concanavalin A, in young mice always of approximately the same age at the time they were used for an assay. What has been seen is a rhythm for each of these measurements that is circannual and phased in a lunar cycle. In general, most responses of mouse splenic lymphocytes are reduced during the winter season and rise in January and February. These recurring circannual changes appear to be endogenous. A rhythmic pattern of reactivity may help explain the variability one sees in the results obtained over a period of time. Whether the rhythmicity persists in all ages of mice is not known.

Studies on the maternal influence on the immune function of the offspring have shown that in 75% of the new born there are maternal cells in the liver. The effects of these cells and their identification as to type is not known. The maternal cells are detected with Fluorescein labelled anti-histocompatibility antisera, using a technique of embryo transplantation with allogeneic combinations.

The studies of T cell memory in a cytotoxicity assay system were slowed by an unexplained decrease in functional ability of cells from our young animals. This problem made our control data suspect while not really changing the data obtained using cells from the old mice. The project will need to be evaluated when this problem is eliminated. The same problem affected the mitogen studies, in that, the viability of the cultured cells decreased rapidly over a 4 day period and made it impossible to continue the secondary stimulus experiments.



The effect of thymic hormone on the generation of membrane markers for T cells has been initiated to determine if changes in T cell function can be induced in old mice.

Significance to Biomedical Research and the Program of the Institute:  
We are gaining a better definition and appreciation of the term immunodeficiency. Since a relative immunodeficiency is seen in aging, it is important to develop better diagnostic criteria, so that possible remedial measures can be undertaken.

Proposed Course: To continue to outline the connection between form and function and to expand our technical ability to measure functions. To develop better diagnostic criteria and tests to describe immune capacity.

**Publications:**

Nordin, A. A. and Adler, W. H.: The effect of aging on in vitro cellular interactions. Fifth Annual Irwin Strassburger Memorial Seminar on Immunology. Developmental Immunology, in press.

Adler, W. H. and Chrest, F. J.: The mitogen assay as a measure of the immune deficiency of aging mice. Fifth Annual Irwin Strassburger Memorial Seminar on Immunology. Developmental Immunology, in press.

Adler, W. H., Jones, K. H. and Nariuchi, H.: Ageing and Immune Function. In Thompson, R.A. (Ed.): Recent Advances in Clinical Immunology. Edinburgh, London and New York, Churchill Livingstone, 1977, pp. 77-100.

Nariuchi, H. and Adler, W. H.: Endotoxin stimulation of antibody production by mouse spleen cells. J. Immunology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00096-05-CPB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less)  Low Temperature Effects on Cells of Aging Individuals										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">M. A. Brock</td> <td style="width: 33%;">Research Biologist</td> <td style="width: 33%;">CPB NIA</td> </tr> <tr> <td>Other:</td> <td>W. H. Adler</td> <td>Medical Officer, PHS</td> <td>CPB NIA</td> </tr> </table>			PI:	M. A. Brock	Research Biologist	CPB NIA	Other:	W. H. Adler	Medical Officer, PHS	CPB NIA
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Other:	W. H. Adler	Medical Officer, PHS	CPB NIA							
COOPERATING UNITS (if any)  None										
LAB/BRANCH Gerontology Research Center, Clinical Physiology Branch										
SECTION Clinical Immunology Section										
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224										
TOTAL MANYEARS: 1.0	PROFESSIONAL: .9	OTHER: .1								
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SUMMARY OF WORK (200 words or less - underline keywords)  The objectives of this study are to characterize possible <u>age-related</u> differences in the <u>in vitro</u> responses of murine <u>lymphohemopoietic cells</u> to <u>mitogen</u> and their differential susceptibility to <u>freezing</u> damage. <u>Murine splenic lymphocytes</u> were subjected to a broad range of cooling rates using a <u>micro-computer controlled rate controller</u> , held at $-196^{\circ}\text{C}$ , rapidly thawed, and tested for functional activity. The incorporation of $^3\text{H}$ -thymidine was used to assess cellular proliferation after <u>in vitro</u> activation of the lymphocytes by various <u>mitogens</u> . A high percentage of functionally unimpaired cells was recovered at cooling rates from $-0.25^{\circ}\text{C}$ to $-5.0^{\circ}\text{C}/\text{min.}$ , a broader range of rates than previously reported for murine and human lymphocytes.										
GRC/CPB-102										

## Project Description:

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in (1) the in vitro responses of human and murine lymphohemopoietic cells to various agents and (2) the differential susceptibility of murine and human immunocompetent cells to freezing damage assessed by their functional recovery after thawing.

Methods Employed: The percentage of viable cells in suspensions was determined using the stains, fluorescein diacetate and ethidium bromide to identify viable cells and non-viable cells, respectively. Suspensions of murine lymphocytes containing  $15$  or  $30 \times 10^6$  cells/ml in RPMI-1640 with 10% DMSO and 10% fetal calf serum were frozen using cooling rates ranging from  $-0.25^\circ\text{C}$  to  $-5.0^\circ\text{C}/\text{min}$ . At  $-50.0^\circ\text{C}$ , the glass vials containing the frozen lymphocytes were transferred to liquid  $\text{N}_2$ . The cells were later thawed rapidly, and their viability and functional recovery compared to control unfrozen cells. The incorporation of  $^3\text{H}$ -thymidine into DNA was used to assess the mitotic activity of the cells after in vitro activation of T-lymphocytes by the mitogens, phytohemagglutinin (PHA) and Concanavalin A, (Con A) and B-lymphocytes by lipopolysaccharide (LPS).

Major Findings: The microcomputer-controlled rate controller described previously that was used to cool suspensions of murine lymphocytes provided a constant cooling rate that apparently conferred protection on the cells over a broader range of rates than reported for murine and human lymphocytes. From 70 to 100% of the unfrozen cells were recovered after cooling at rates between  $-0.5^\circ\text{C}$  and  $-5.0^\circ\text{C}/\text{min}$ . Functional recovery of T- and B-lymphocytes as assessed by the incorporation of  $^3\text{H}$ -thymidine after activation by PHA and LPS was similar over a range of cooling rates from  $-0.25^\circ\text{C}$  to  $-5.0^\circ\text{C}/\text{min}$ . T-cells activated by Con A and cooled at  $-0.25^\circ\text{C}$  and  $-0.5^\circ\text{C}/\text{min}$  incorporated 80% and 90%, respectively, of the activity of cells cooled at rates of  $-1.0^\circ\text{C}$  to  $-5.0^\circ\text{C}/\text{min}$ . Compared with unfrozen, control cells, the frozen-thawed cells incorporated 80-100% of control levels of  $^3\text{H}$ -thymidine at cooling rates ranging from  $-0.25^\circ\text{C}$  to  $-5.0^\circ\text{C}/\text{min}$ .

Several modifications were made to the cooling chamber in order to increase the cooling efficiency of the liquid  $\text{N}_2$  vapor entering the chamber. The structural changes combined with the programmable rate controller provided a constant cooling rate, rapid sensing of the heat of fusion released as extracellular ice crystallized, and automation of additional solenoid valves to permit rapid entry of liquid  $\text{N}_2$  vapor, with restoration of the programmed cooling rate.

Significance to Biomedical Research and the Program of the Institute: The reported decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an in vitro system which tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. Controlled rate cooling is a new technique that may be used to preserve lymphocytes for further study at the cellular level or for therapeutic use.

Proposed Course: The characterization of possible age-related changes in mammalian lymphoid cells will include further studies on the enhancement of splenic lymphocytic responses to mitogens as these may be related to cellular biomembrane systems. Age-related lymphocytic resistance to stress will be tested using controlled rate freezing techniques.

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-AG-00104-02-CPB																								
PERIOD COVERED <b>October 1, 1977 to September 30, 1978</b>																										
TITLE OF PROJECT (80 characters or less) <b>Clinical Immune Survey of the Longitudinal Project Participants</b>																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. H. Adler</td> <td style="width: 35%;">Medical Officer, PHS</td> <td style="width: 15%;">CPB NIA</td> </tr> <tr> <td>Other:</td> <td>A. A. Nordin</td> <td>Research Chemist</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>K. H. Jones</td> <td>Staff Fellow</td> <td>CPB NIA DOD 7/15/78</td> </tr> <tr> <td></td> <td>J. E. Nagel</td> <td>Clinical Associate, PHS</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>P. L. Mann</td> <td>Visiting Fellow</td> <td>CPB NIA</td> </tr> <tr> <td></td> <td>R. K. Saxena</td> <td>Visiting Fellow</td> <td>CPB NIA EOD 7/1/78</td> </tr> </table>			PI:	W. H. Adler	Medical Officer, PHS	CPB NIA	Other:	A. A. Nordin	Research Chemist	CPB NIA		K. H. Jones	Staff Fellow	CPB NIA DOD 7/15/78		J. E. Nagel	Clinical Associate, PHS	CPB NIA		P. L. Mann	Visiting Fellow	CPB NIA		R. K. Saxena	Visiting Fellow	CPB NIA EOD 7/1/78
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TOTAL MANYEARS: <b>4.1</b>	PROFESSIONAL: <b>2.3</b>	OTHER: <b>1.8</b>																								
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SUMMARY OF WORK (200 words or less - underline keywords) Over the past year 200 individuals in the <u>Longitudinal Program</u> have been included in the survey of <u>immune function</u> . On a cross sectional basis the preliminary findings are the <u>"T" cell function</u> as measured by proliferative and cytotoxicity assays starts to <u>decrease</u> in the <u>5th decade</u> of life. <u>Antibody production</u> , a B cell assay, <u>remains stable</u> throughout life. A nonspecific host defense system, the <u>granulocyte function</u> drops off with age beginning at the 4th decade. <u>Lymphocyte numbers</u> in the <u>peripheral blood</u> start a steady age related decline starting at the 3rd decade. In the coming year, we will begin to restudy individuals for the second time to determine the stability of the results obtained this year.																										

GRC/CPB-105

## Project Description:

Objectives: The purpose of this project is to assess different immune functions and to correlate these results in order to gain an overall picture of the individuals' immunologic ability. Since there are many assays that can be performed, we will present each individually with the understanding that through the correlational analysis of the data we will be able to arrive at the most useful assays to employ in measuring human immune function.

The three broad areas of this program will be data collection, research and service. The data collection and research aspects will be dealt with specifically in terms of investigation of serum antibody and lymphocyte responses. The service aspect will be in terms of a consultative function in setting up assays that use immunological methods, such as radioimmune assays for hormones, or fluorescent antibody staining in histopathology.

The specific assays of human immune function which will be used in this study are as follows.

1. The measurement of serum immunoglobulin levels by radial-diffusion.
2. The measurement of specific antibody to immunogens, such as diphtheria or tetanus toxins, and pathogens, such as pneumococci, influenza, E. coli, etc.
3. The measurement of peripheral blood granulocyte function.
4. The identification and quantitation of subpopulations of lymphoid cells in the peripheral blood.
5. One assay of immune lymphocyte function will be the response of lymphocytes to mitogens for T cells (PHA, Con A), B cells (PWM, SA), and antigens (Diphth Tox, Tet Tox, Candida). This test will measure proliferative ability in both the primary exposure and secondary exposure.
6. The measurement of the T cell-helper effect will be incorporated into the assay systems used to evaluate immune function. The necessity of thymus-derived lymphocytes (T cells) to interact with bone marrow-derived lymphocytes (B cells) to produce antibody to most antigens has been well established. Since there is an impressive amount of evidence that T cell function declines with age, it is not unreasonable to suspect that the decline of antibody synthesis with age is a result of a lack or malfunction of this helper cell activity associated with T cells.
7. Another assay will be the determination of the number of lymphocytes in peripheral blood which can differentiate to antibody-forming cells. There is evidence to indicate that immunoglobulin synthesis in aging mice and humans becomes restricted and often converts to a monoclonal nature. However, it is not yet appreciated if such a restriction in any way affects the integrity of the immune system.

8. The measurement of Epstein-Barr virus (EBV) infection will be accomplished by determining if cells from older humans will form established EBV infected lymphoblast cell lines in tissue culture. We will also determine the presence of anti-EBV antibody in the serum as evidence of exposure.
9. The determination of the activity of peripheral blood T lymphocytes in cytotoxic assay systems against human and mouse tumor cells in vitro. This is a measure of spontaneous T cell killing which can be useful in determining cellular population changes and T cell function.

Methods Employed:

1. Lymphoid cell preparations:
  - a. Human peripheral blood lymphocytes (PBL) will be isolated from freshly drawn heparinized blood of the participants of various age groups in the program. The blood is diluted with an equal volume of tissue culture medium carefully layered over Ficoll-Hypaque and the tube centrifuged. The lymphocytes are retained at the interface and are easily collected. The cells are thoroughly washed to remove any Ficoll-Hypaque and resuspended in a tissue culture medium. These cells will be identified and cultured in standard systems.
2. Tissue culture and assay:
  - a. The human PBL are mixed at various concentrations with a fixed number of murine B cells and cultured for four days. The antigen used to stimulate the cultures will be sheep erythrocytes.
  - b. The detection of antibody-producing cells will be by the plaque assay which allows a quantitative estimate of the number of cells actually synthesizing antibody. Therefore, we can titrate the helper cell effect and establish the level of the effect as opposed to an "all or none" type of information.
  - c. The detection of cytotoxic T lymphocyte activity will be by chromium isotope labelling of the target tumor cells and then incubation with the lymphocytes. The measure of isotope release by the target cells will be the measure of the cytotoxicity induced by the lymphocytes.
3. Assays of immunoglobulin synthesizing cells will be accomplished by plating pokeweed-stimulated blood lymphocyte cultures with sheep red cells that have been coated with Protein A. The development of plaques will be accomplished using specific antisera and a complement source.
4. EBV titers will be accomplished by standard methods using lymphoblast cell lines, serum titration and a fluorescent anti-immunoglobulin antisera.

Major Findings: To date 200 individuals have been examined using the various assays described above. All these people were examined for the first time and during the next year we will begin to examine some of them for the second time to see if assay results will change over a period of time.

The results so far, interpreted in a cross-sectional analysis of the aging effect on immune function demonstrate:

1. No age effect on the antibody forming ability of the peripheral blood lymphocytes.
2. An age related decrease in granulocyte metabolic function with an onset in the 4th decade.
3. An age related decrease in T cell proliferative response to mitogens with the first changes seen at the 5th decade and marked change at the 6th decade.
4. No change in serum immunoprotein levels.
5. An age related decrease in T cell cytotoxic activity with an onset in the 5th decade.
6. At this time the data on the morphologic determination of cell types in peripheral blood in terms of T cells and B cells has not been done, but analysis of lymphocyte levels has shown a steady decline of lymphocyte numbers after the second decade.
7. T cell helper assays are just being initiated.
8. A study of the pokeweed mitogen induced immunoglobulin production (PFC's) and proliferation (thymidine incorporation) of human lymphocytes showed that the number of PFC's was not correlated to proliferation in the cultures. Furthermore, the amount of pokeweed needed for immunoprotein production was much lower in concentration than the amount needed for proliferation. This study will be the basis of an analysis of function of lymphocytes from donors of different ages.
9. The study of lymphocyte nucleic acid synthetic pathways using the inhibitor chloramphenicol has demonstrated that lymphocytes do not metabolize the drug, the cell viability is directly related to drug concentration, the suppressive effect of the drug on nucleic acid synthesis is most pronounced if the drug is added at the initiation of the culture, it has no effect on cells which have already entered a lymphoblast phase, and cells from older individuals are much more affected by the suppressive effects of the drug than are cells from younger people. These results may help in determining cellular metabolic changes seen with age.



Significance to Biomedical Research and the Program of the Institute:

There is no doubt that these studies, along with the many other pieces of information available on this population, will yield valuable data to show the presence or absence of a correlation of aging diseases and disorders with a deficiency of immune function. At present, there is no information on this subject. If we find that an age-associated immunodeficiency does lead to specific diseases, we could, hopefully, avoid the disorders by augmenting immune function. It may also be possible to alter our immunogens so as to make them more effective in the aging host.

Proposed Course: The original intention of this project is to follow the subjects in the Longitudinal Program to ascertain the changes seen in their immune function and the effects this might have on their patterns of illness.

Publications: None

NIA ANNUAL REPORT  
October 1, 1977 through September 30, 1978  
Gerontology Research Center  
Laboratory of Behavioral Sciences

During the past year this laboratory has made a number of significant administrative and programmatic advances. Authority has been granted for the creation and establishment of a new Section on Stress and Coping. Recruitment of a chief of this section is now in progress. There has been an expansion of collaborative research programs with non NIA scientists. In addition to the continuing collaborative activities which have existed between investigators in LBS and various divisions in Baltimore City Hospital, there now are projects including investigators from Columbia Medical Plan, the Human Development Program of the University of Maryland and the Department of Psychology of the University of Baltimore. In conjunction with these program developments, there has been an expansion of the non-tenured scientific staff to include fellows supported through the Intergovernmental Personnel Act and the Visiting Fellow Program. In addition, there are guest workers from various universities who receive no salary support from NIA. The interest among so many scientists in the programs of LBS reflects both the importance of those programs to the scientific community at large, and the growing interest among scientists in the behavioral problems of middle aged and elderly adults. In this regard it should be noted that this laboratory has become one of the leading research centers in the world in the rapidly developing field of Behavioral Medicine: the application of behavioral principles and techniques to the care and treatment of medical patients.

There are several clinical research projects which have been completed this year. One project was designed to determine if patients with angina pectoris could be trained to slow their heart rates, and to determine if such training could affect the patients' performance during an exercise stress test. The results of this study were that all six patients were able to learn to slow their heart rates consistently in the laboratory, and four of them showed a post-training reduction during an exercise stress test. Overall results for all six patients were: increase in time on treadmill, 37%, 2.05 min; increase in work on treadmill, 108%, 1442 kg. m/min. Another study was designed to identify some of the factors which characterize patients who sign out from a cardiac care unit against medical advice. The findings were that patients who signed out against medical advice had less evidence of cardiac disease and fewer deaths in the post-hospitalization period, more self-reports of alcohol abuse and of emotional disturbances, and they manifested more behavior problems while in the hospital. The next study is a follow-up to a project reported several years ago. It was reported then that patients with fecal incontinence could be trained to become continent by learning to emit synchronized, phasic contractions of their internal and external anal sphincters. One puzzling aspect of that result was the nature of the phasic external sphincter contraction. If, as was commonly believed, this was a reflex response, how could patients learn to control it? The present research clarified that issue by showing that the phasic contraction of the external anal sphincter is not a reflex; it is a voluntary response. A normal subject is able to inhibit the sphincteric contraction immediately

upon instruction to do so. Patients with histories of chronic constipation, who do not experience strong urges to move their bowels, and who have had fewer occasions during which they were required to emit external sphincteric responses, are less likely to emit sphincteric responses to a standardized rectal stimulus, and the responses that they do emit are weaker than those of normal subjects.

A major focus of research in this laboratory is on the way the central nervous system operates to modulate sensory and motor processes. It is widely accepted that in man auditory information is processed sequentially; i.e., paired stimuli arriving simultaneously at each ear are reported as though they had been presented first to one ear and then to the other. However, most investigators believe that visual information is processed simultaneously; i.e., paired stimuli arriving simultaneously at each eye are reported to appear as simultaneous. Research in this laboratory has now shown that if paired digits are presented to the two visual fields at very short durations (75msec), some people will report them as though they were presented sequentially; i.e., these subjects will report the numbers as pairs presented first to one visual field and then to the other. These findings are important because they suggest that there is one general principle underlying all information processing, and because they offer an objective means of assessing age changes or differences in information processing. The hippocampus is a neocortical structure lying within the temporal lobe which has been shown to be important in the mediation of spatial learning. However, there is considerable controversy in the literature as to whether the hippocampus also is important for other movement-related behaviors, and whether electrical activity in the hippocampus not only signals movement but also reward-related aspects of the task. Research completed this year in this laboratory shows that electrical activity in the 4-8 Hz band, recorded from the hippocampi of monkeys reflects both movement and success/failure related aspects of the task. Specifically, correlations of 4-8 Hz activity with heart rate and movement, indicate that generally as the monkey increases its heart rate, it increases its movement and the prevalence of 4-8 Hz activity. However, if one trains the animal to slow its heart rate, and then compares the density of 4-8 Hz activity during periods when the animal is slowing its heart rate conditionally, to periods when the animal slows its heart rate a comparable amount, but spontaneously rather than under the condition of contingent reward, one will find that under contingent conditions the animal will emit significantly more 4-8 Hz activity than under spontaneous conditions. These data suggest that the hippocampus reflects both motor activity and success/failure aspects of the task. The contingency factor is normally masked under conditions where movement also occurs. However, contingent heart rate slowing is a useful task because it reveals an increase in hippocampal activity under conditions where movement is reduced.

Finally, there have been several projects in this laboratory that have explored age-related changes in a variety of functions. One project examined memory and age in the Baltimore Longitudinal Study Program. This study showed that there was a decline in memory for designs (Benton Visual Retention Test) among men in their seventies. Furthermore, by use of an original statistical model, it was possible to show that this longitudinal decline in memory was independent of any secular changes which also might have been



present. It is likely that this model will prove very useful in the analysis of other data from the Baltimore Longitudinal Program. Cross-sectional research on age differences in electrodermal activity point more and more to the conclusion that as subjects grow older, the ratio of the epidermal skin potential to the sweat gland potential increases. Although the data are not yet conclusive, the results suggest that the main age effect is a decline in the sweat gland potential. This inference is based on the observation that age differences in sweat gland potential occur only with high epidermal resistances, because under usual recording conditions young adults have a lower ratio of epidermal resistance to sweat gland resistance than do older men. Thus, under these conditions the sweat gland potential will make relatively less contribution to the recorded potential of the young adults than it will to the recorded potential of old adults. Studies of the dopaminergic, neurotransmitter system of the nigrostriatum of the rat have revealed important, age-related effects: The evidence points strongly to the conclusion that older rats may have a defect in the pre-synaptic, nigrostriatal system. Previous experiments had shown that amphetamine-induced turning behavior is stronger in young rats with unilateral nigrostriatal lesions than it is in old rats so treated. Current findings are that there are no age differences in turning behavior when the dopaminergic agonist, apomorphine is injected into lesioned animals; that there is a 33% reduction in dopaminergic receptor binding in the old animal; and that L-DOPA potentiates amphetamine-induced turning behavior in the young rat but not in the old animal. Thus, all of these data point to an age-related deficit in pre-synaptic functioning. A major project on the interaction between exercise and diet was completed this year. This project found that among genetically homozygous mice (C57B1/6J), voluntary wheel exercise late in life increased longevity. Likewise, survival was directly proportional to dietary protein (low = 5%, normal = 26% and high = 48%) late in life. But what was especially interesting was that these two effects interacted multiplicatively so that the animals which received the high protein diet and the opportunity to engage in voluntary exercise lived longest and maintained their body weights at higher levels than did animals fed low protein diets, or animals without wheel exercise. These data suggest that protein diet and exercise may be important factors in maintaining health and vigor late in life.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00061-16 LBS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Behavioral Genetics and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles L. Goodrick

Research Psychologist LBS GRC NIA

OTHERS: Teena M. Wax

Guest Worker

LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center - LBS

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Md. 21224

TOTAL MANYEARS:

1.10

PROFESSIONAL:

.50

OTHER:

.60

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to determine behavioral differences throughout the lifespan for populations of mice which differ in genetic structure, and which also differ in longevity. The present topics of interest concern the relations of growth rate, maximal body weight and longevity for mice fed low levels of dietary protein and for groups fed adequate dietary protein, in addition to studies of aging and voluntary wheel exercise, and ethanol preference.

GRC/LBS-113

Project Description:

Objectives: The principal objectives of this project are: (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., over-dominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Old age is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating  $F_2$  hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets with 4% (low protein), 26% (control), and 48% (high protein) casein are used. Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories; Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations. In addition, inbred and hybrid mice are used to test the hypothesis that protein malnourishment increases longevity due to slowing of the rate of growth. Studies of behavioral differences as a function of dietary protein and stage of development are also in progress.

Major Findings: Although mice fed low protein diets beginning at an early age have longer life spans than mice fed normal protein diets, the effect of changing dietary protein later in the life span has not been studied. Aged (24 month old) male C57BL/6J mice ( $N = 135$ ) were allowed voluntary wheel exercise ( $n = 75$ ) or were maintained in standard cages as controls ( $n = 60$ ). All mice were switched to isocaloric synthetic diets with the protein level the same as the normal laboratory diet, i.e., 26%. After 3 weeks, 1/3 of each group were placed on a low diet protein (4%), 1/3 were placed on a high protein diet (48%), and 1/3 remained on the normal protein diet. For all three groups allowed voluntary wheel exercise (for a total of 9 weeks) amount of voluntary wheel exercise was positively correlated with life span. Voluntary wheel exercise resulted in an increase in life span compared with controls, and this effect was greater with increasing dietary protein. The mean life span was also a directly increasing function of dietary protein. Mice fed high protein diets engaged in more voluntary wheel exercise, lived longer, and maintained body weight at a higher level

compared with mice fed low protein diets. A high level of dietary protein may be an important factor in maintaining health and vigor late in the life span.

2. A typical finding with respect to various types of obese rodents has been that, for these animals, amount of voluntary exercise is very low compared with nonobese controls.

The purpose of the present research was to determine behavioral motivational differences between genetically obese mice and control mice, where body weight was held at a relatively low, constant level for all mice by feeding once per day. Tests of voluntary wheel exercise were conducted over a longer time interval than previously, and during bar pressing tests, the number of rewards for each mouse was the same, an uncontrolled factor in previous research. In addition, the present research studied differences in the ability of genetically obese mice and control mice to solve a complex problem to obtain a food reward and to discriminate low concentrations of sucrose.

In the first experiment, genetically obese mice and controls kept at a constant weight were maintained in exercise wheels for a period of 7 weeks starting at 5 weeks of age. The hypothesis was tested that the obese group would be inactive relative to the control group, an adaptive response which would facilitate body weight increment.

Mean body weight and mean daily wheel activity for the seven weeks are given in Table 1 for the obese and control groups. The obese mice were significantly higher in body weight than the control mice, with no overlap between the two groups. Voluntary wheel exercise (shown in Table 1) was not significantly different for the two groups, but obese mice were significantly more active than control mice on the last three weeks of testing, Group X Weeks,  $F(6,48) = 2.63$ ,  $p < .05$ .

In previous experiments, obese animals made fewer bar-press responses on fixed-interval reward schedules, while obese animals had similar or greater response levels than controls on fixed-ratio reward schedules. The next part of the experiment was designed to study responses within a fixed interval (FI) of 80 sec. where all mice received the same number of rewards in the same time interval. During this period of daily testing, when the mice were 14-15 weeks old, body weight group differences were highly statistically significant,  $F(1,14) = 143.4$ ,  $p < .01$ , Table 2, with the obese group maintained at 27-30 gm. and the control group maintained at 17-19 gm. The control mice made significantly more responses on the neutral bar than the obese mice,  $F(1,14) = 5.56$ ,  $p < .05$ , and because of the variability of these differences over trials, the interaction of group and trials was also statistically significant,  $F(4,56) = 5.44$ ,  $p < .01$ , Table 2. Responses on the reward bar were significantly greater for obese mice than for control mice,  $F(1,14) = 7.84$ ,  $p < .05$ . When the 80 sec. interval was divided into 20 sec. quadrants, the quadrants closer to the reward had proportionally more responses,  $F(3,42) = 88.27$ ,  $p < .01$ . The group X FI quadrant inter-



action was highly statistically significant,  $F(3,42) = 20.08$ ,  $p < .01$ ; group differences were small in the first two 20" quadrants, but obese mice averaged nearly twice the responses of control mice during the two quadrants prior to the reward.

Problem-solving tasks can provide both performance and learning measures, but such tasks have been used rarely in the study of motivational differences between obese animals and control animals where there are group differences in body size or age. In this part of the study, obese mice and control mice were trained to run a 14-unit T-maze to obtain a food reward. Performance measures of time to reach the reward and learning measures of errors were recorded. Obese mice made significantly fewer errors than control mice,  $F(1,14) = 5.44$ ,  $p < .01$ , errors decreased over trials,  $F(15,210) = 49.56$ ,  $p < .01$ , and the group X trials interaction was significant,  $F(15,210) = 1.97$ ,  $p < .02$ , because group differences were reduced over trials. For the time measure, the groups were not different. Time to reach the reward was significantly reduced over trials,  $F(15,210) = 31.31$ ,  $p < .01$ . The group X trials interaction was significant,  $F(15,210) = 1.99$ ,  $p < .02$ ; the obese mice obtained the food reward faster than control mice on the initial trials, but obese mice were slower than control mice on the last trials.

Experiments from other laboratories found that food deprived rats were better able to discriminate sucrose solutions than controls, and drank more sucrose solution than controls. The present study examined the ability of obese mice to discriminate low concentrations of sucrose and also examined the amount of fluid ingested during discrimination testing. It was hypothesized that obese mice would ingest more fluid than controls and would drink proportionally more sucrose solution than water in the 2-bottle tests, compared with control mice. During these 96-hour tests, the mice could select their fluid from one of two tubes on the front of their cage. One tube always contained water, while the other contained water plus sucrose (1, 0.5, 0.25, .125 or .063 gm sucrose/100 ml.).

Control mice ingested more fluid per 96 hr. test interval than obese mice,  $F(1,14) = 19.45$ ,  $p < .01$ , and fluid intake decreased as a function of sucrose concentration,  $F(5,70) = 100.95$ ,  $p < .01$ . Because the group differences were reduced at the low sucrose concentrations, the interaction of group X concentration was also statistically significant,  $F(5,70) = 6.24$ ,  $p < .01$ .

Control mice ingested a greater percentage of sucrose solution than obese mice, but the difference was not statistically significant,  $F(1,14) = 3.35$ ,  $p > .05$ . Percentage of sucrose solution declined as a function of decreasing sucrose concentration,  $F(4,56) = 70.90$ ,  $p < .01$ , and the group X concentration interaction was significant because control mice ingested a significantly greater percentage of sucrose solution at a low .125 gm sucrose/100 ml concentration indicating better discrimination of the sucrose by control mice than for obese mice.



In summary, genetically obese mice and control mice were maintained at a constant low level of body weight (about 50% of maximal weight) with all of the obese mice heavier than the control mice. The genetically obese mice were more active, made more bar presses to obtain a food reward, and made fewer errors initially during learning of a complex problem than control mice. If increased levels of voluntary wheel exercise, increased rates of bar pressing during a fixed interval, and faster learning are indices of high motivation level, then obese mice were more food motivated than control mice. It was concluded from the results of these tests that obesity in these mice does not result from inactivity. The obese mice have the option of remaining inactive to gain weight, but they do not behave in this way. Although obese mice were more highly food motivated than control mice, final level of learning for food rewards and sensory discrimination of sucrose for obese mice were not greater compared with control mice.

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied; (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world population. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Further inbred strains and  $F_1$  hybrid groups are being studied to determine the generality of mode of inheritance of behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with various mouse strains. The longevity of inbred and hybrid groups are also being determined. Experiments with low, normal, and high protein diets should determine: (1) the effect of varying protein diets upon behavior at maturity after access to these diets during various stages of development, and (2) the immediate effects upon behavior of a diet of low, normal or high protein for young or aged mice.

Publications:

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Goodrick, C. Ethanol preference of inbred mice: Mode of inheritance and the effect of age on the genetic system. Journal of Studies on Alcohol. 1978, 39, 19-38.

Wax, T. & Goodrick, C. Nearness to death and wheelrunning behavior in mice. Experimental Gerontology, In Press.

Table 1  
 Mean Body Weight and Voluntary Wheel Exercise Over a 7-week  
 Period for Obese Mice and Control Mice

Group	Weeks						
	1	2	3	4	5	6	7
	BODY WEIGHT (gm.)						
obese	26.5	26.6	22.0	23.7	24.7	24.8	24.4
control	18.0	16.5	16.9	18.3	17.9	16.3	16.1
	DAILY WHEEL EXERCISE (rev/day)						
obese	6,794	9,000	9,017	7,111	10,314	10,609	8,086
control	8,406	10,320	7,431	6,737	8,717	7,617	5,440

Table 2

Mean Body Weight, Mean Total Neutral Bar Press Responses, and Mean Total  
 Reward Bar Responses as a Function of Trials for Obese Mice and  
 Control Mice on a Fixed Interval (80") Reward Schedule

	Trials				
	1	2	3	4	5
	Body Weight (gm.)				
obese	28.8	28.3	27.4	27.9	29.0
control	18.2	17.5	17.1	17.5	18.2
	Neutral bar				
obese	104	170	124	101	119
control	207	197	178	307	239
	Reward bar				
obese	1,626	1,585	1,511	1,483	1,339
control	1,048	1,157	953	1,018	874





Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, various races, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of a structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, nightdreams, fantasies, etc. There are 28 scales in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

In a vigilance task, the subject must detect changes in visually or auditorially presented material. For example, the subject may see a stimulus designated "A" flash on a projection screen at a specific rate; sometimes, however, another stimulus designated "B" will occur instead of A. The subject's task is to detect and report when B appears. The proportion of B's can be controlled as well as the number of stimuli presented per minute and the interval at which stimuli remain in view. These factors determine the difficulty and tediousness of the vigilance task and are expected to be related to the incidence of daydreaming.

Major Findings:

I. Racial, Religious, ethnic, and socio-economic effects on daydreaming. Data from more than 1800 people who responded to the Imaginal Processes Inventory and to a biographical questionnaire have undergone a preliminary analysis to determine unconfounded differences due to religious, racial, ethnic, and socio-economic differences. Presently inferential statistical analyses are being carried out to determine whether any differences observed in the preliminary analyses are reliable.

II. Interrelations among daydreaming characteristics, health, and estrogen usage in pre-menopausal, menopausal, and post-menopausal women. A prior study of sex differences in daydreaming (see 1976-77 annual report) found a large drop in likelihood of sexual daydreaming in women at age 50 years as compared with women 35 to 49 years. Since age 50 is the menopausal transition period for most women, it was decided to investigate this potential relationship more closely. Information on daydreaming, menopausal state, health, demographic variables, and drug usage has been collected on 476 women 40 to 60 years of age and is being analyzed.

III. Vigilance and daydreaming incidence.

The vigilance task described earlier will be used to measure directly the incidence of daydreaming for people across the life-span. A life-span sample will be used since the self-reports of subjects suggests that with

increasing age there is an increased ability to concentrate on any task without the intrusion of daydreams. Presently, equipment necessary to carry out this project is being designed and constructed.

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed course of project

1. Starting in 1979, men in the Baltimore Longitudinal Study will be asked to complete the Imaginal Processes Inventory. For most men in study, this will constitute a second completion of the Imaginal Processes Inventory. The time interval since first completion for most men will have been 6 years and thus information will be obtained on intra-individual age changes in daydreaming.
2. A factor analysis of the items of the Imaginal Processes Inventory will be carried out on the responses of the more than 1800 people who completed it. The factor analysis will result in a set of daydreaming characteristics which may be considered as each separately representing a separate and independent dimension of daydreaming. These factors will then be used to re-examine the effect of previously examined variables' -- including age -- on daydreaming.
3. The study relating vigilance, boredom, and daydreaming will be carried out on a life-span sample.

Publications:

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Giambra, L. M. A factor analytic study of daydreaming, imaginal process, and temperament: A replication on an adult male life-span sample. Journal of Gerontology, 1977, 12, 675-680.

Z01-AG 00062-05 LBS

Giambra, L. M., & Traynor, T. D. Depression and daydreaming: An analysis based on self-ratings. Journal of Clinical Psychology, 1978, 34, 14-25.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00063-11 LBS																				
PERIOD COVERED October 1, 1977 through September 30, 1978.																						
TITLE OF PROJECT (80 characters or less)  Learned Modification of Visceral Function in Animals																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">J. A. Joseph</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 30%;">LBS, GRC, NIA</td> </tr> <tr> <td>Other:</td> <td>B. T. Engel</td> <td>Chief, LBS</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>G. S. Roth</td> <td>Research Chemist, Endocrinology</td> <td>CPB, GRC, NIA</td> </tr> <tr> <td></td> <td>C. Filburn</td> <td>Research Chemist</td> <td>LMA, GRC, NIA</td> </tr> <tr> <td></td> <td>S. P. Tzankoff</td> <td>Research Physiologist</td> <td>CPB, GRC, NIA</td> </tr> </table>			PI:	J. A. Joseph	Senior Staff Fellow	LBS, GRC, NIA	Other:	B. T. Engel	Chief, LBS	LBS, GRC, NIA		G. S. Roth	Research Chemist, Endocrinology	CPB, GRC, NIA		C. Filburn	Research Chemist	LMA, GRC, NIA		S. P. Tzankoff	Research Physiologist	CPB, GRC, NIA
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COOPERATING UNITS (if any)																						
LAB/BRANCH Laboratory of Behavioral Sciences																						
SECTION Psychophysiology																						
INSTITUTE AND LOCATION GRC, NIA, NIH, Baltimore, Maryland 21224																						
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SUMMARY OF WORK (200 words or less - underline keywords)																						
<p>           The purpose of this project is to investigate the role of the <u>central nervous system</u> in <u>behavior</u>. In some experiments we examine the contribution of <u>somatic factors</u> in <u>instrumental cardiovascular conditioning</u>. Brain areas of special interest are the <u>hippocampus</u>, the <u>caudate nucleus</u> and the <u>pyramidal tract</u>; subjects are <u>monkeys</u> (<u>Macaca mulatta</u>). In other experiments we examine the age-related changes in <u>pre- and post synaptic</u> portions of the <u>nigrostriatal pathways</u> using <u>behavioral</u> and <u>biochemical</u> analytical methods. The present topics of interest include possible alterations in: (a) development of <u>post synaptic denervation hypersensitivity</u>; (b) specificity and affinity of <u>dopamine receptor</u> binding; (c) <u>adenyl cyclase</u> production; and (d) <u>synaptic</u> release mechanisms that might occur with age.         </p>																						

GRC/LBS-125

## Objectives

A. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

B. To analyze the age differences in biochemical and structural characteristics of the brain in relation to behavior.

## Methods Employed

A. In numerous experiments, in a variety of lower animals, the hippocampus, a neocortical structure, has been implicated in somatic control. This structure has a dominant frequency of 4-8 Hz which can be recorded from the pyramidal cell layer of the dorsal hippocampus when the animal performs or intends to perform a voluntary movement. This frequency has been designated as "theta" activity or rhythmic slow activity (T). As a first attempt at deducing some of the possible CNS mechanisms that might be involved in the instrumental control of heart rate, electrodes were chronically, bilaterally implanted in the hippocampi, pyramidal tracts and caudate nuclei of monkeys operantly conditioned to speed and to slow their heart rates. In one animal (6) the electrodes were implanted prior to training to assess T early in conditioning. Since the hippocampus seems to be related to movement, and since heart rate changes might be related to changes in movement, more theta activity should be seen during heart rate speeding sessions than during slowing sessions. Each session comprised a 512 sec baseline period and a 2048 sec testing period. Three types of sessions were examined: "speeding sessions" during which the animal was operantly trained to speed its heart, "slowing sessions" during which the animal was trained to slow its heart, and no feedback sessions in which the animal was not required to speed or to slow its heart. It simply sat in the closed booth throughout a 512 sec baseline period and a 2048 sec "training" period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow its heart and a green light indicated the speeding condition. A white light functioned as a reinforcement light to indicate to the animal that it was performing correctly. A 10 ma, .45 sec electric shock was delivered to the tail once/8 sec for incorrect responding. Speeding and slowing sessions were further subdivided into "push" and "non push" sessions. Push sessions were designated as those in which the animal was required to change its heart rate until it was at some specified level below (slow) or above (speed) its baseline heart rate (10, 15, or 20%). During non-push sessions this was not done; the animal only had to keep its heart rate below or above baseline. For purposes of analysis the sessions were divided into 16 blocks of 128 sec duration and the baseline was divided into 4 blocks of 128 sec duration. Heart rate (HR), systolic (SBP), and diastolic blood pressure (DBP) were recorded as well as samples of hippocampal EEG for the first 10 sec of each block. The "raw" hippocampal activity was filtered by a band pass filter over the 4 to 8 Hz range and then integrated. The activity in this range was defined as theta (T) and the amount of theta for each 128 second block was computed. Additionally a fast Fourier analysis

was carried out on the 10 sec samples of hippocampal activity so that the energy distribution from 1-50 Hz could be examined. Gross motor activity (M) was scored visually by a trained observer during the first 10 sec. of each 128 sec block.

B. It has been reported previously that a surgically induced lesion in the substantia nigra of young animals results in a substantial decline in the level of a dopamine (DA) in the caudate nucleus on the lesioned side. The caudate nucleus has been shown to be very important in the control of movement, and an imbalance in the neurotransmitters between the lesioned and unlesioned nigrostriatal pathways produces a directional dominance in the animals' movements such that after injection of certain drugs such as apomorphine (a DA receptor agonist) or amphetamine (which promotes the release of DA) animals can be induced to turn in circles. These rotations can be accurately measured with a device called a rotometer. Numerous studies have shown that: (a) amount of depletion; (b) DA receptor activity; (c) development of denervation receptor hypersensitivity; and (d) presynaptic release events can be gauged by the strength and directionality of the rotational behavior. This behavior seems to be almost exclusively modulated by the nigrostriatal system and by dopaminergic-cholinergic interactions within this system. In the behavioral experiments lesions are produced unilaterally (left side) in the substantia nigra of young (4-6 mo) and old (25-29 mo) male and female rats following several prelesion baseline measures of rotational behavior. Following a 7-10 day recovery period baseline rotations are again examined to determine if there are any effects of surgery. After this the animals are given a graded series of doses of: (A) amphetamine (0.5, 1, 2, 5 mg/kg); (B) apomorphine (0.25, 2, 5 mg/kg); and (C) apomorphine (5mg/kg) followed by haloperidol (a dopamine receptor antagonist) (.25, .5, 1, 2 mg/kg). Each animal receives all doses of all drugs (2 doses at each level), but it receives the complete series of one drug before being given the next. In other experiments young and old animals are given amphetamine prior to lesioning to determine if a natural imbalance in dopamine exists between the left and right caudate nuclei and if this imbalance is enhanced or diminished with age. In a third set of experiments changes in the numbers of dopamine receptors in the caudate nuclei are assessed through binding studies carried out with tritiated haloperidol and some assessment also is made of adenylyl cyclase, phosphodiesterase, and tyrosine hydroxylase in the caudate nuclei of young and old rats. In a fourth group of experiments the rotational behavioral method is used to examine what pharmacological agents might be utilized to overcome deficits in turning behavior seen in old animals. Old and young animals (rats) are given 1 mg/kg of amphetamine on alternate days (3 doses/wk) for 40-50 days after surgery. Very stable response rates are achieved with this method. They are then given 3 graded doses of L-DOPA alone (2, 3, 5 mg/kg) on alternate days. Then they are given the 5 mg/kg dose of L-DOPA 1 hr. before a 1 mg/kg dose of amphetamine is given. They are tested for 1/2 hour. This is replicated 3 times. Dopamine levels are assayed in the left and right striata from these animals to determine the efficacy of the lesion. A parallel group of lesioned old and young animals is used to assess striatal dopamine levels 1 hr. after an L-DOPA injection. Dopa decarboxylase and phosphodiesterase is assessed in still another group of old and young animals.



Major Findings

A. The data for the push and non-push sessions have been considered together for purposes of this analysis. Each experimental period was divided into 16, 128 sec. blocks, and the blocks were analyzed according to the following criteria: speeding, blocks in which the animal increased HR >5 beats/min from the previous block (F); and blocks in which the animal speeded less than 5 beats/min from the previous block (f); slowing, in which the animal decreased HR >5 beats/min (S); and in which the animal decreased HR <5 beats/min (s). In all cases the heart rate block selected had to be above (speeding) or below baseline (slowing) in order to be considered. Blocks also were selected in a similar manner from the control (no feedback) sessions (Fn, fn, Sn, sn, respectively). Correlational analyses revealed (Kendall's coefficient of concordance, W; Spearman's rank order correlation, R) that there was a high degree of consistency among animals in each response measure across experimental conditions (Ws HR = .98, SBP = .97, DBP = .97, T = .98, M = .68). Theta also was related to increases in heart rate. The highest percentage of theta usually occurred during contingent and non-contingent speeding (Table 1). Thus there tended to be very high rank order correlations between HR and T for all animals (6, R = .97; 7A, R = .71 7B, R = .78).

Table 1

Mean percent theta ( $\pm$  std error of the mean) for the different contingent and non-contingent training conditions

Condition	Animal #6	Animal #7A	Animal #7B
F	7.4 $\pm$ 1.1	3.5 $\pm$ 1.3	8.1 $\pm$ 1.9
Fn	10.3 $\pm$ 1.4	8.9 $\pm$ 2.5	6.6 $\pm$ 1.9
f	1.2 $\pm$ 0.6	1.5 $\pm$ 0.7	-0.1 $\pm$ 0.1
fn	3.5 $\pm$ 1.0	0.1 $\pm$ 1.2	2.7 $\pm$ 0.2
s	1.2 $\pm$ 0.4	0.9 $\pm$ 1.0	2.9 $\pm$ 1.2
sn	-1.4 $\pm$ 0.7	0.0 $\pm$ .93	-1.7 $\pm$ 0.9
S	-4.5 $\pm$ 1.3	2.3 $\pm$ 3.1	1.5 $\pm$ 4.0
Sn	-11.6 $\pm$ 1.7	-7.8 $\pm$ 2.1	-7.3 $\pm$ 1.4

One interesting finding that emerged from this analysis is that while T showed a direct relationship to HR for most conditions, the animal always produced more T under the contingent slowing conditions than the non contingent slowing conditions (e.g., S vs Sn  $\bar{X}$  Diff, 7A = 10.06,  $t = 2.77$   $p < .01$ , 7B = 8.78  $t = 2.64$   $p < .02$ , 6 = 5.97  $t = 2.54$   $p < .02$ ). This same phenomenon was not seen when the difference scores for motor activity under these same conditions were analyzed. It was seen that there were very little differences among contingent and non-contingent slowing sessions and M. This suggests that T, which in most cases was closely related to M (R, 6 = .83; 7A = .73; 7B = .30) and HR (see above) becomes dissociated from somatic activity during contingent slowing. Moreover, it is further suggested that T may be reflecting both motor and success/ failure related components. During contingent speeding the success/ failure correlation is masked by concomitant motor activity (M and HR, R 6 = .88, 7A = .90, 7B = .40), however, during



contingency slowing this correlation is revealed because M is reduced while T is increased. These relationships do not change significantly over the course of conditioning, since analysis of animal 6's early conditioning data revealed similar trends to those seen late in conditioning. Thus, the relationships of T, M, and HR seem to be highly stable both within and between animals. Analysis of the data from the pyramidal tracts and caudate nuclei has not been completed but a preliminary analysis of the power density functions from the pyramidal tracts of animal 7B indicates a slight shift into the 1-3Hz band during HR speeding sessions.

B. Since all animals (32; 8 old males, 8 young males, 9 old females, 7 young females) were lesioned in the left substantia nigra, DA levels in the left caudate nucleus should be diminished. When amphetamine is given, DA release is promoted on the unlesioned side and the animal turns toward the left. It has been shown in young animals that the greater the strength of turning toward the lesioned side the greater the imbalance in DA that exists between the lesioned and unlesioned striatum. Results of the analysis of baseline turning rates showed that old animals emitted less overall turning than did young animals ( $\bar{X}$  = 2.0 turns/30 min, old;  $\bar{X}$  = 16.5 turns/30 min, young). Under the influence of amphetamine the animals began to turn increasingly toward the left as a function of dose. This occurred in both old and young animals. A direct comparison of the magnitude of the differences in left turn behavior with increasing doses of amphetamine was difficult to make because there were age differences in the initial turning behavior prior to the drug injections. Therefore, the data were transformed and a strength of response measure was computed by taking the left turns for each animal and dividing them by the right turns. Baselines were equated (old .88, young .85) and the resulting analysis of variance showed that old animals emitted lower ratios of left/right turns, and that this was true throughout the dose range ( $\bar{X}$  dose ratio overall: Old = 5.5, young = 24.0). There were no differences between males and females in response strength. Old animals tended to increase both right and left turns as the dose of amphetamine was increased, while young animals selectively increased left turns. These findings suggest that there is a defect in DA responsivity at either the pre or post synaptic receptor on the unlesioned side since histological analysis of the brain of these animals showed that the lesions were all of the same size. It is difficult to say where the deficit lies, but on the basis of the apomorphine data discussed below it is likely that the deficit is in the releasing mechanism. Since apomorphine is a DA receptor agonist, it should affect both caudate nuclei if injected intraperitoneally. Since the caudate on the lesioned side develops degeneration hypersensitivity apomorphine should affect it more strongly, and the animal should exhibit contralateral turning (i.e., away from the lesion side) behavior. A deficit in: (a) development of hypersensitivity, or (b) receptor number or both should be reflected in diminished responding. An analysis of variance computed on the ratios of the contralateral to ipsilateral turns showed that there was no age effect ( $F < 1.0$ ) even though the drug increased turning behavior ( $F > 4.0$ ,  $p < .0001$ ). These findings suggest that the deficit seen in the old animals shown earlier probably is mediated presynaptically. Additionally, these findings could mean that there are natural deficits in release

of dopamine in old animals in which case the receptors may be in a constant state of hypersensitivity so that any decrease in numbers will not be seen with direct agonist stimulation. A subsequent assay has revealed a 33% decrease in DA receptor binding in the old animal. Evidence for this deficit in presynaptic mediation of rotational behavior can be found from data in our laboratory in which L-DOPA potentiation of amphetamine induced turning occurred in young rats but not in the senescent rat. Young rats increased their left/right turn ratios by 50% while old rats showed a 23% decline in the ratios. Subsequent neurochemical analyses have tended to support these behavioral findings in that both the levels of dopamine and tyrosine hydroxylase, (the rate limiting step in the synthesis of dopamine) are reduced. The young rats showed a  $10.9 \pm .7$  mg/kg (mean  $\pm$  S.D.) DA in the unlesioned striatum and a  $1.8 \pm .7$  ug/g DA in the lesioned striatum while the old rats showed  $5.12 \pm .7$  and  $2.03 \pm .7$  in these areas respectively. Thus, old rats showed a 50% reduction in DA from that of young rats in the unlesioned striata. Moreover, a 12-17% depression in both stimulated and unstimulated tyrosine hydroxylase activity was seen in the striata of the senescent rats. Data from the dopa decarboxylase, adenylyl cyclase, and phosphodiesterase determinations have not yet been analyzed since a larger number of animals is needed to complete the assessments.

#### Significance to Bio-medical Research and the Program at the Institute

There is growing evidence that the central nervous system imposes a high degree of plasticity on peripheral motor functions. Thus, responses which ordinarily occur in one way under one set of conditions, may occur in another way under another set of conditions. Two processes which operate upon the nervous system to modulate peripheral function are learning and aging. Until we understand fully how these processes operate, and under what conditions they will express themselves, we will never be able to explain behavior in normal or diseased individuals, or in subjects of any age. Furthermore, when we understand the principles which underlie neurally mediated plasticity, we should be able to evolve strategies which will enable subjects to compensate, at least in part for the infirmities associated with the disorders of adult life. For example, the neural systems that we are now studying in the primate project deal with the integration of somatomuscular and cardiovascular responses. It is well-known that both of these motor systems become less adaptable with age, but what is not known is the extent to which this diminished adaptability can be modified by experience. Another example of the relevance of this project to the assessment of age-dependent neural changes in plasticity comes from the work on the nigrostriatal system. It is well-known that neuromuscular disorders of late adult life such as Parkinsonism are associated with various changes in function and structure of the nigrostriatal system. It is our goal to determine the extent to which these age-related declines can be ameliorated.

Proposed Course of the Project

A. The present experiment will be continued so that the contributions of the hippocampus during instrumental heart rate conditioning can be assessed more definitively. Other CNS areas concerned with somatic control will be examined during cardiovascular conditioning in order to determine their involvement in mediating this response. In conjunction with this, data will be collected on the effects of stimulation of various hypothalamic, medial midbrain reticular formation, and posterior hypothalamic pressor, depressor, cardioacceleratory and deceleratory areas will be examined under the three "training" procedures, i.e., fast, slow, and no feedback, in order to see if the effects of stimulation can be altered by conditioning: For example, if stimulation in a particular anterior hypothalamic area is found to produce pressor and acceleratory cardiovascular changes: Would these changes be ameliorated when the monkey is asked to slow its heart? How would pressor/ acceleratory stimulation interact with operant speeding performance? Finally, we will place constraints on operant heart rate conditioning through exercise. The chaired monkey will be trained to pull a 10 kg weight attached to a lever on a fixed ratio FR(8) schedule. Heart rate and blood pressure will be recorded. The animal will then be trained to slow and to speed its HR in the manner described above. Attempts will then be made to determine if such training influences performance on the exercise task. For example, can the monkey, after it has been trained to slow HR, learn to exhibit a lower HR while exercising, and is the converse also true? By these experiments we can specify more clearly some of the central and somatic mediational components of heart rate control.

B. So far the project has concentrated on the dopamine system. Further investigations will examine turning behavior after modifications on serotonergic, noradrenergic, and cholinergic systems. In addition, control studies will be carried out to determine other factors that might account for the differences in turning behavior with age (e.g., differences in the rate of metabolizing test drugs, etc.).

Publications

Joseph, J. A., Appel, J. B: Behavioral sensitivity to LSD: Dependency on the pattern of serotonin depletion. Pharmacol. Biochem. Behav. 6: 499-504, 1977.

Joseph, J. A., Berger, R. E., Engel, B. T. and Roth, G. S.: Age related changes in the nigrostriatum; A behavioral and biochemical analysis. J. Gerontol., in press.

Engel, B. T.: Somatic mediation of heart rate: A physiological analysis. In Proceedings of the First International Meeting on Biofeedback and Self-Regulation, Tübingen, Germany, in press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00064-17 LBS								
PERIOD COVERED <p style="text-align: center;">October 1, 1977 to September 30, 1978</p>										
TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Problem Solving and Aging</p>										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PIs:</td> <td style="width: 30%;">David Arenberg,</td> <td style="width: 30%;">Chief, Learning &amp; Problem Solving Section</td> <td style="width: 30%;">LBS GRC NIA</td> </tr> <tr> <td></td> <td>Leonard M. Giambra</td> <td>Research Psychologist</td> <td>LBS GRC NIA</td> </tr> </table>			PIs:	David Arenberg,	Chief, Learning & Problem Solving Section	LBS GRC NIA		Leonard M. Giambra	Research Psychologist	LBS GRC NIA
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SECTION <p style="text-align: center;">Learning and Problem Solving Section</p>										
INSTITUTE AND LOCATION <p style="text-align: center;">NIA, NIH, Baltimore, Md. 21224</p>										
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SUMMARY OF WORK (200 words or less - underline keywords) <p>The primary purposes of this project on <u>aging</u> are to; (1) identify <u>reasoning processes</u>; (2) determine how these processes <u>change</u> with age; and (3) develop techniques for reducing <u>age deficits</u> in reasoning performance. Reasoning is studied using <u>problem solving</u> procedures including <u>concept learning</u> and <u>concept identification</u>.</p>										

GRC/LBS-132



Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: All of the three experiments in progress are long-term aging studies. Experiments IV and V are longitudinal studies; and in Experiment XI each subject solves more than 100 problems and a model is constructed which describes and predicts how he or she solves a wide variety of concept-learning problems.

Experiment IV, a study of logical problem solving, was designed so that the analytic aspects of the problem are independent of the synthetic aspects. Each problem is solved in two separate parts. In the first part, the task is to obtain all the pieces of information to solve the problem (analysis); in the second part, all the correct pieces of information are provided, and the task is to use that information to solve the problem (synthesis). In previous research of this kind, analytic and synthetic performance were confounded; errors of analysis could appear to be difficulty in synthesis of information. The separation of the two aspects of the task provide unfounded measures of analytic and synthetic performance.

Experiment V is a longitudinal study of concept identification. In this study, subjects select instances (examples) sequentially and are informed whether each selection is a positive example of the concept or a negative example. On the basis of the information elicited by these selections and their classification, the specific attributes of the concept can be identified. This procedure provides a measure of information gain for each selection. Ordinarily when subjects select instances, logically equivalent selections do not always result in equivalent information gains, and fortuitous selections can alter drastically the difficulty of a problem. A technique was developed which provides equivalent information gains for logically equivalent selections and avoids fortuitous gains in information. This technique also has two other important characteristics: (1) different types of problems which are logically equivalent can be constructed and compared, e.g., conjunctive concepts in which two attributes must both be present can be matched with two-attribute disjunctive concepts in which either attribute can be present; and (2) initial information gain can be manipulated because the experimenter can minimize or maximize the information gain for each selection. Experiment V was designed to determine age differences and age changes for six different types of problems determined by the number of attributes in the concept (one or two), and for two-

attribute problems, the concept rule (conjunctive and disjunctive) and the amount of initial information gain.

Experiment XI is a concept study in which each subject solves a large number of problems. The literature in concept identification is based almost entirely on mean effects of subsamples in which each person solves one or a few problems. It has not been established that the variables which affect the mean performance of groups would affect an individual in the same way. When a person solves one or a few problems, his approach to reasoning can be characterized as unstable, variable, transient, and highly subject to chance occurrences. Solving many problems is expected to result in a steady state of performance. In that steady state, strategies can be more readily elicited and the effects of various independent variables on changes in strategy and on other performance measures can be studied. After solving 48 complete learning problems, and again after 96, 8 more problems are solved in which subjects "think out loud" throughout each problem. When individuals "think out loud" they are verbalizing all the thoughts about the concept problem that are in their conscious awareness. They are specifically asked: (a) to relate the basis of their concept classification of the stimulus instance before them; (b) to tell if they had seen the stimulus instance earlier in the problem and if they remember its concept classification; (c) to relate how they use the feedback information about the correct classification of the stimulus instance before them to help them learn the concept; and (d) for any mnemonics they use to remember any and all aspects of a problem during the solution. The protocols of these "thinking-out-loud" problems constitute the primary data of this study. They are used to construct individual models of how a subject solves complex concept problems with which he is highly practiced. These models have as a general goal the prediction of each individual's performance on concept learning problems by specifying at each point in a problem how the individual will classify a stimulus instance as an exemplar or a nonexemplar of the concept. This general goal is met by succeeding at the more fundamental goals of specifying: (a) what information and past stimulus instances are stored in memory as well as for how long and what the retrieval cues are; and (b) how information on the exemplar status of a stimulus instance is utilized, inductively or deductively, to arrive at a solution to the problem, i.e., to identify the unknown concept. To achieve goals (a) and (b) a detailed specification of mechanisms is needed. These finely detailed mechanisms, appropriately connected in a flow diagram, constitute the individual's model. Comparisons of these mechanisms and their connections provide the means of specifying individual differences as well as age (generational) differences.

Major Findings: Experiment IV is the longitudinal study of logical problem solving. Cross-sectional analyses of partial data were reported previously. No further analyses were carried out this year.

Experiment V is the longitudinal study of concept problem solving. Preliminary cross-sectional and longitudinal results were reported previously. No new analyses were carried out this year.

Experiment XI is the study in which each subject solves a large number of concept-learning problems. Data collection is complete for 11 men (ages 12, 18, 20, 26, 40, 54, 63, 72, 87, 96, and 106 years) and 11 women (ages 13, 18, 26, 35, 48, 51, 66, 74, 81, 89, and 90 years). A model has been specified for three men (ages 18, 63, and 96), and a model for the 90 year old woman is partially specified. Automated apparatus was constructed to provide time measures of all aspects of concept learning.

Significance to Bio-Medical Research and the Program of the Institute:

Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection will continue for all three studies in progress. Longitudinal data through June, 1979, for Experiment V will be analyzed; a manuscript will include cross-sectional and conventional longitudinal analyses, and, if feasible, sequential analyses (developed in this laboratory for data collected continually) will be applied to selected performance variables. Longitudinal data collection for Experiment IV is expected to be completed in about two years at which time the study will be terminated. A longitudinal study of problem solving in women will be initiated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00065-18 LBS									
PERIOD COVERED October 1, 1977 to September 30, 1978											
TITLE OF PROJECT (80 characters or less) Verbal Learning and Age											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: David Arenberg                      Chief, Learning & Problem                      LBS GRC NIA Solving Section  Others: None											
COOPERATING UNITS (if any)  <table style="width: 100%;"> <tr> <td style="width: 33%;">E.A. Robertson-Tchabo</td> <td style="width: 33%;">Baltimore City Hospitals</td> <td style="width: 33%;"></td> </tr> <tr> <td>L. Potash</td> <td>University of Maryland</td> <td></td> </tr> <tr> <td></td> <td>University of Maryland</td> <td></td> </tr> </table>			E.A. Robertson-Tchabo	Baltimore City Hospitals		L. Potash	University of Maryland			University of Maryland	
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L. Potash	University of Maryland										
	University of Maryland										
LAB/BRANCH Gerontology Research Center - Laboratory of Behavioral Sciences											
SECTION Learning and Problem Solving											
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Md. 21224											
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CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  The primary purposes of this project on <u>aging</u> are to identify measures of <u>verbal learning</u> and <u>memory</u> which change with <u>age</u> , to specify <u>psychological processes</u> and their relationships with age, and to develop <u>procedures for improving learning and memory performance</u> in the elderly.											

GRC/LBS-136



Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiment XXVIII was a study of the effects of ethanol infusion on several aspects of cognitive performance. One of the tasks was delayed recognition memory. Twenty-four words were presented sequentially, and the subject's task was to say "Yes" (meaning "I saw this word in the list of twelve presented a few minutes ago.") or "No" (meaning "I did not see this word in that list."). Analyses of correctness measures have been reported previously. Recently, error data were analyzed to determine the effects of ethanol infusion on response bias.

Experiment XXXII is a study of the effects of propranolol on learning and information processing. Learning is measured by a multi-trial, free-recall procedure. In addition, simple reaction time is included along with four choice-reaction-time tasks in which the cognitive load is varied. Subjects receive an injection of propranolol or saline (double blind--neither the subject nor the experimenter knows what is injected). Several hours before and two hours after the injection, all performance measures and concurrent heart rate recordings are obtained. Two aspects of this study are: (1) the effects of propranolol on performance in young (28-41 years) and old (61-80 years) men, and (2) the relationship among heart rate, cognitive load, and performance in young and old men.

Experiment XXXIV is a memory study in which proactive interference is used to explore age differences in semantic encoding. When several lists of the same category (e.g., all occupations) are to be remembered one after the other, recall declines over lists. This effect is called proactive inhibition, i.e., interference from preceding activities. When a list of a different category (e.g., fruits) follows several lists of the same category, (e.g., occupations) recall improves substantially. This effect is called release from proactive inhibition. One hypothesis which has been supported by a few age studies is that the old process semantic information less effectively than young adults; and, therefore, memory tasks which are dependent on semantic encoding should result in large age differences in performance. Age deficits in semantic encoding are investigated in this study by varying the amount of semantic similarity between categories in the proactive-release procedure. The three age groups are: (1) 20 - 39 (2) 60 - 69, and (3) over 70.

Experiment XXXV is an age study of free recall in which some items (words) in a list are presented two or three times. For some words, the repetitions are contiguous (massed), for others, the repetitions are separated by several other words (distributed). Previous research has demonstrated that, for young adults, words with distributed repetitions are more likely to

be recalled than words with massed repetitions. Animal studies in this laboratory, however, have demonstrated that complex maze learning is enhanced for old rats (but not for young rats) when learning trials are massed. The current study is designed to explore whether massed practice (in the sense of repetitions of items) is beneficial for recall memory in old adults. Each word is shown for 2 seconds, but the interval between words is varied (1 or 7 seconds) to determine whether time to assimilate the material to be recalled affects the "distribution-of-practice" effects. The two age groups are: (1) 18-25 years, and (2) 60 - 75 years.

Major Findings: Experiment XXVIII was the study of the effects of ethanol on cognitive performance. Errors in recognition memory are of two kinds -- "misses" (failures to recognize previously seen items) and "false positives" (incorrectly "recognizing" items not previously seen). "Misses" increased substantially under acute alcohol regardless of age. In signal detection theory, increases in "misses" without increases in "false positives" are regarded as changes in response bias (rather than in accuracy), i.e., a diminished willingness to say "Yes, I have seen that item before." Typically, this is interpreted as increased cautiousness. The fact that ethanol produced a decreased willingness to say "Yes" is counter to the expected decrease in cautiousness under alcohol. (During infusion, 4 of the 40 subjects responded "No" to every item despite the fact that they knew that 12 of the 24 items had been seen a few minutes earlier. It is possible, however, that reduced willingness to say "Yes" is not an indication of cautiousness in this situation, but rather of cognizance of severely impaired delayed memory. In this forced-choice task subjects are required to say either "Yes, I have seen that word in the most recent list" or "No, I have not seen that word in the list." When subjects are confronted with this forced choice under alcohol, the awareness of impaired memory (high uncertainty) apparently leads to an increased tendency to say "No." The data are clear that response bias in delayed memory is affected substantially by ethanol, but it seems questionable to interpret the decreased willingness to say "Yes" as increased cautiousness in the usual sense of that word. Under alcohol, when forced to respond the men apparently preferred "misses" to "false positives."

Experiment XXXII is the study of learning and information processing in young and old men before and after propranolol (or saline). The results based upon 13 young experimental subjects, 10 old experimental subjects, and 6 old control (saline) subjects were similar to the preliminary findings from the learning data reported last year; propranolol had no effect. As in previous studies (in this laboratory) using these response-time tasks, age differences were found in the time measures, and these differences were largest for the most complex tasks. In addition, the old made more decision errors (predominantly missed signals) than the young in the complex tasks.

Experiment XXXIV is the memory study of semantic encoding using release from proactive interference. Analyses of data are in progress. It is clear that release from proactive inhibition occurred in both old groups (in addition to the young adults). The patterns of magnitudes of release,

however, were systematic over the similarity dimension only for the young groups. As a result, it may be difficult to interpret the findings with regard to the semantic encoding hypothesis.

Experiment XXXV is the free-recall study of massed vs. distributed practice. Apparatus has been constructed, procedures have been developed, and data collection has been initiated.

Significance to Bio-Medical Research and the Program of the Institute:

Memory and learning are central to experimental psychology, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning and memory of the older person.

Proposed Course of Project: Distribution-of-practice will be applied to other types of learning and memory, possibly motor-skill learning. If the results confirm that the old benefit from massed practice, procedures will be developed to explore the processes underlying this general finding. Further efforts will be made to adapt laboratory findings to develop procedures for improving memory and learning skills for the old. Relationships between blood pressure and levels and changes in learning measures of longitudinal participants will be explored in collaboration with the Clinical Physiology Branch.

Publication:

Arenberg, D. Introduction to a symposium: Toward comprehensive programs for memory problems among the aged. Experimental Aging Research, in press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00066-17 LBS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Perceptual Retention and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David Arenberg

Chief, Learning and  
Problem Solving Section

LBS GRC NIA

OTHER: None

COOPERATING UNITS (if any) Baltimore City Hospitals

Elizabeth A. Robertson-Tchabo, University of Maryland

Don Reynolds, University of Baltimore

LAB/BRANCH

Gerontology Research Center, Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

1.9

PROFESSIONAL:

.6

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are: (1) to investigate perceptual retention and interference; (2) to determine under what conditions age differences in retention are affected by interference; and (3) to investigate processes of interference and perception. Current studies include nonverbal memory, a visual analog of the dichotic-listening procedure, and massed vs. distributed practice in simple reaction time and decision time.

GRC/LBS-140



Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VII is a longitudinal study of memory for designs in which subjects attempt to reproduce visual designs from memory. The Benton Visual Retention Test is used, and the primary dependent measure is the total number of errors in all ten designs. Each design consists of geometric figures presented for ten seconds and then withdrawn. The task is to reproduce each design from memory. Subjects may take as much time as they need to draw the design.

Experiment X is a study of visual information processing using a visual analog of the dichotic-listening procedure. Verbal information is generally believed to be processed sequentially by the auditory system and simultaneously by the visual system. The dichotic-listening procedure, in which pairs of different stimuli are presented simultaneously (one to each ear) and two or more pairs are presented sequentially, has been used to demonstrate that auditory information is processed sequentially. Under specific timing conditions, many people report such stimuli ear by ear rather than by simultaneous pairs (as presented). A visual analog of the dichotic-listening procedure was devised in this Section to determine whether, under specific timing conditions, visually presented stimuli are reported sequentially (visual field by visual field) rather than by simultaneous pairs. Each simultaneous pair consists of two different digits, one in the left visual field and one in the right.

Experiment XI is an age study of massed vs. distributed practice in simple reaction time and decision time. Animal studies in this laboratory have shown that old rats (unlike young mature rats) learn a complex maze with fewer errors when trials are close together in time (massed) than when trials are several hours (or a day) apart. Experiment XI is designed to determine whether massed practice is beneficial to old humans on response-time tasks which are expected to show improvement in performance over trials. Two tasks are used: (1) simple reaction time -- during a 90 sec. period, a zero appears 15 times on a screen, and the subject presses a hand-held button as quickly as he can; and (2) choice reaction time (decision time) -- 90 digits are presented sequentially on a screen at a one-second rate, a designated digit appears 15 times, and the subject monitors the screen and presses the same button as quickly as he can every time the designated digit appears. Two age groups are included: (1) 18-25 years and (2) 60-75 years. For the five trials (90 sec. periods), the time from the beginning of one trial to the beginning of the next is either two minutes or one hour.

Major Findings: Experiment VII is the longitudinal study of memory for designs (Benton Visual Retention Test). Analyses of two-point data with an interval of six or more years were previously reported. The primary finding was the relation between age and change in performance. Only the men initially in their seventies had substantial increases in mean errors. Three-point data with an interval of twelve or more years between first and third measures are now available for the men initially tested between 1960 and 1964 (and who continued in the study). The groups initially under 60 continued to show little or no change. The group initially in their sixties, however, had a substantial increase in mean errors from the second to the third measure. These data are consistent with the two-point data for the men initially in their seventies. When the 60-69 group was retested six or more years after their initial performance, these men were in their late sixties or early seventies. The increase in mean errors was small. When the same group was tested a third time, the men were in their middle or late seventies or early eighties. Then they were about the ages of the men initially in their seventies when they were retested six or more years later, and it was then that the substantial increase in errors was found.

When the two-point data were analyzed and showed substantial increases in mean errors for the men initially tested when they were 70-79, there was some suggestion of secular effects which could have contributed to these longitudinal changes. This suggestion was based upon comparisons of men who entered the study at different times but were the same age when first tested. For some age groups, mean errors for the men who entered the study between 1968 and 1973 were somewhat greater than mean errors for the men who entered the study between 1960 and 1964. A regression procedure was developed in this Section to estimate whether the changes were largely attributable to secular changes or were attributable, at least in part, to maturation. With this regression procedure, it was found that the longitudinal increases in errors for the men initially tested when they were in their seventies was largely attributable to aging rather than to secular change.

Experiment X is the study of information processing using a visual analog of the dichotic-listening procedure. Pilot data indicated that at the very short duration of 75 msec for each stimulus exposure (with no inter-stimulus interval), several individuals reported sequential pairs, i.e., the two digits presented in one visual field (and projecting to one cerebral hemisphere) followed by the two digits presented to the other visual field (and projecting to the other hemisphere). This preliminary finding suggests that the visual system processes verbal information sequentially under specifiable timing conditions.

Experiment XI is the response-time study of massed and distributed practice. Data collection has begun.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea,

however, is sparse and not consistent. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. In addition, response slowing is a general behavior which pervades many aspects of information processing; another purpose of this project is to improve our understanding of information processing and how it changes with age.

Proposed Course of Project: If sequential responding is found at short exposures (75-0-75 msc) but not at somewhat longer exposures, one important question is whether the sequential responding occurs only when the two exposures are perceived as simultaneous, i.e., the two pairs of digits are seen as one exposure of four digits. Age comparisons are planned, also. Important research questions include: (1) Are some older individuals unable to report simultaneous digits (similar to the findings for dichotic digits)? If so, are the same individuals unable to process simultaneous information by both the visual and auditory systems? and (2) Do the old respond sequentially at longer exposure periods than young? If so, are longer exposures perceived as simultaneous? Is this related to the psychological refractory period? In addition, relationships between scales of the Guilford-Zimmerman Temperament Survey and memory will be explored.

Publications:

Arenberg, D. Differences and changes with age in the Benton Visual Retention Test. Journal of Gerontology, 1978, 33: 534-540.

Douglas, K. and Arenberg, D. Age changes, cohort differences, and cultural change on the Guilford-Zimmerman Temperament Survey. Journal of Gerontology, 1978, 33: 737-747.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> <b>INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 AG 00067-11 LBS																																												
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<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">B. T. Engel</td> <td style="width: 30%;">Chief, LBS</td> <td style="width: 20%;">LBS, GRC, NIA</td> </tr> <tr> <td>Other:</td> <td>K. Gaarder</td> <td>IPA Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>M. Garwood</td> <td>Staff Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>M. Glasgow</td> <td>IPA Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>J. McCroskery</td> <td>IPA Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>A. Perski</td> <td>Visiting Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>E. Lakatta</td> <td>Chief, Cardiovascular Section</td> <td>CPB, GRC, NIA</td> </tr> <tr> <td></td> <td>S. Gottlieb</td> <td>Cardiologist</td> <td>Balto City Hospital</td> </tr> <tr> <td></td> <td>W. Baile</td> <td>Staff Fellow</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>W. Whitehead</td> <td>Guest Worker</td> <td>LBS, GRC, NIA</td> </tr> <tr> <td></td> <td>M. Schuster</td> <td>Gastroenterologist</td> <td>Balto. City Hospital</td> </tr> </table>			PI:	B. T. Engel	Chief, LBS	LBS, GRC, NIA	Other:	K. Gaarder	IPA Fellow	LBS, GRC, NIA		M. Garwood	Staff Fellow	LBS, GRC, NIA		M. Glasgow	IPA Fellow	LBS, GRC, NIA		J. McCroskery	IPA Fellow	LBS, GRC, NIA		A. Perski	Visiting Fellow	LBS, GRC, NIA		E. Lakatta	Chief, Cardiovascular Section	CPB, GRC, NIA		S. Gottlieb	Cardiologist	Balto City Hospital		W. Baile	Staff Fellow	LBS, GRC, NIA		W. Whitehead	Guest Worker	LBS, GRC, NIA		M. Schuster	Gastroenterologist	Balto. City Hospital
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SUMMARY OF WORK (200 words or less - underline keywords)  <p>           This project is concerned with the interaction of <u>behavior</u> and <u>physiology</u> in <u>man</u>. One study investigates behavioral procedures (<u>relaxation</u> and <u>biofeed-back</u>) in the control of <u>blood pressure</u> in patients with <u>high blood pressure</u>; a second study investigates <u>heart rate</u> biofeedback in patients with <u>angina pectoris</u>; two studies investigate <u>medical non-compliance</u> in patients with <u>histories of cardiac disease</u>; two studies investigate <u>bowel function</u> in normal man and patients with <u>irritable bowel syndrome</u>; one study investigates <u>age differences in autonomic response patterns</u>; one study investigates age differences in <u>electrodermal activity</u>.         </p>																																														
GRC/LBS-144																																														



Project Description:

Objectives

A. To evaluate the clinical effectiveness of relaxation and biofeedback in the control of blood pressure in hypertensive patients.

B. To determine if patients with angina pectoris can learn to slow their heart rates, and can transfer this skill to an exercise stress test.

C. To learn if non-compliance behavior among patients with recent myocardial infarctions can be made compliant through the use of specific behavioral interventions.

D. To identify the characteristics of patients who sign out of a coronary care unit against medical advice.

E. To determine whether patients with clinically significant urinary incontinence characterized by uninhibited neurogenic bladders or by urinary sphincter dysynergia can be trained to recover control of their bladder/sphincter functions.

F. To evaluate behavior modification and bowel biofeedback in the control of the signs and symptoms associated with irritable bowel syndrome.

G. To determine the functional properties of the external anal sphincter. Specifically, to learn if the phasic contraction of the external anal sphincter following brief rectal distension is a reflex or a voluntary response.

H. To describe age differences in individual patterns of autonomic responses.

I. To identify the mechanisms underlying age differences in electrodermal activity.

Methods Employed:

A. Patients are selected from the outpatient service of the Columbia Medical Plan. All patients will be diagnosed as suffering from high blood pressure. Study patients will be limited to those who are receiving no antihypertensive medication and who have clinic diastolic pressures  $\geq 90$  mm Hg; and patients who are receiving diuretic therapy (only) for their hypertension regardless of their clinic pressures. Approximately 100 patients will participate in a multiphasic, controlled study. Phase 1 will be one month long and will be a baseline phase. All patients will record their blood pressures 9 times/day (3 times upon awakening; 3 times during the middle of the day; three times during the evening). In addition, patients will have their pressures taken approximately once weekly by a health professional. In phase 2 patients will be assigned to one of three groups: Self administered relaxation, self administered blood pressure biofeedback,

control. Assignment to groups in phase 2 will be such that the three groups are matched in blood pressures, and such that approximately equal numbers of patients in each group will be either untreated or will be taking diuretics. Phase 2 will be 3 months long. Phase 3 will be similar to phase 2 in terms of duration and nature of the treatments: control patients will continue; relaxation patients will be subdivided into two groups--group A will continue to relax, group B will be trained in the feedback condition; feedback patients from phase 2 will be divided into two groups similarly to the relaxation patients. In phase 4 control patients will receive six months of therapy (relaxation followed by feedback); patients on diuretic medication will have their diuretics withdrawn if their diastolic pressures  $\leq 86$  mm Hg, and they will be followed as before; patients who have received only one therapy (relaxation or feedback) will be offered the other treatment; patients who have received both therapies will continue to be followed for 6 months. All patients in phases 2-4 will continue to record their pressures daily, and also will have their pressures taken weekly by a health professional.

B. Six patient were trained in the laboratory to lower their heart rates using well-established procedures which were developed in this laboratory and which have been described in previous reports. In addition, all patients received exercise stress tests twice prior to laboratory training and once following training. Patients also monitored their anginal episodes daily and reported these data daily, and patients were monitored during a prescribed course walk carried out once weekly.

C. Subjects were nine middle-aged men hospitalized in a Coronary Care Unit. They were chosen from a group of patients referred by the staff physician or staff nurse who had judged them to be at high risk for non-compliance during the post-MI period. Our selection was based on the findings from an evaluation interview. All patients met one or more of the following criteria; 1) past non-compliance with a similar regimen (N=2); 2) unrealistic expectation of post-infarction work ability (N=3); 3) over-investment in a life-style (work-style) for which a slowing down of activity would be an obstacle to rehabilitation (N=3); 4) actual non-compliance in the CCU (N=3); 5) denial of infarct (expression of doubt as to the diagnosis) (N=4); 6) extreme delay behavior in getting to the hospital (N=2); 7) statement as to un-willingness to comply with the rehabilitation program (N=1). Seven patients agreed to participate in the program. The two who declined continued to behave in the post-infarction period as though they had not had a serious heart attack. Each patient was told about the specific nature of his infarct; and he was shown his enzyme studies and EKG so that he could have an insight into the nature of his MI. He then was requested to plan a hierarchical program of rehabilitation which was appropriate for him. Each program had the following features: 1) There were 9 or 10 steps; 2) The last step was full-recovery as defined by the patient (for most patients this was "return to full-time work"); 3) Each step in the hierarchy was more difficult (i.e., required a greater number of metabolic equivalent units) than the prior step; 4) Every patient was taught to take his pulse and was supplied with forms to record his pulse before and after each activity as well as his symptoms at each level. Patients who began the program in the

hospital (N=6) were seen daily and then weekly on an out-patient basis. When appropriate, activity lists were modified to add new activities. Pulses were checked periodically by the investigator. Criteria for movement to the next level of activity were: (1) a pulse rate change during the activity of 20 bpm or less from baseline; (2) pulse rate <110 bpm for a particular activity; (3) absence of symptoms (chest pain, shortness of breath and fatigue) for a particular level of activity. Results were analyzed in terms of: (1) number of appointments kept per patient; (2) average number pulse recordings/day over time; (3) number of times patient went out of activity level as reported by wife or patient himself; (4) number of appointments kept in clinic; (5) consistency of pulse changes with different activities; (6) comments of patient's clinic physician.

D. Twenty nine patients who had signed out against medical advice (AMA) were identified from the CCU log book at Baltimore City Hospital, and an equal number of controls was generated by selecting charts of the next patient admitted. Statistical comparisons of the two groups were made along the following dimensions: (1) socio-demographic variables: age, sex, race; (2) illness variables: discharge diagnoses and cardiologist ratings of electrocardiographic and historical data; (3) psycho-social variables: self-report of alcohol abuse and emotional illness; (4) AMA variables: length of stay in the hospital, behavioral observations, previous sign-outs; (5) follow up data.

E. Patients with documented, clinically significant urinary incontinence secondary to the following conditions are eligible: cerebral vascular accidents, spinal cord trauma, tumor or malformation, surgery, diabetic neuropathy, and demyelination of the nervous system. The experimental design will be a simple before-and-after method with each patient serving as his own control. There are three phases to the study. Phase I is a diagnostic procedure during which the nature and severity of the bladder and sphincter control will be determined. First there will be an interview to obtain a record of the degree of incontinence in the daily routine. Then, by means of CO<sub>2</sub> cystometry, pressure in the bladder and along the urethra will be obtained. The procedure involves inserting a catheter through the urethra into the bladder. The catheter has two small openings (one at the tip and another 4 cm. back from the tip) for pressure recording on a direct-writing polygraph. Controlled amounts of CO<sub>2</sub> will be introduced into the bladder through the catheter by means of a regulator to give a filling pressure. Before training intra-vesical and intra-urethral pressures will be taken to provide a diagnosis of the nature of the incontinence. Phase 2 is the training procedure. It consists of showing the patient the immediate record of the sphincter responses as they appear on the polygraph for comparison with tracings of normal sphincter responses. In addition to visual feedback the patient will be given verbal praise for successful responding and encouragement to continue modifying these responses. Phase 3 consists of additional training to aid the patient in developing a strong response and in learning to respond independently of the polygraph. As the patient begins to show appropriate sphincter contractions, the filling pressure will be gradually raised so as to require a stronger sphincter response. As the



patient learns to respond to normal filling pressures, feedback will be periodically withheld. Each training session will last approximately two hours, and sessions will be spaced at weekly intervals. The patient will be urged to practice sphincter control between sessions. No patient will receive more than five sessions. One month after the last session the patient will return to the laboratory for a follow-up session to assess retention of sphincter control.

F. The study will involve approximately 20 normal subjects and 20-40 patients with IBS. Patients must meet the following criteria: (1) altered bowel habits, (2) abdominal pain, (3) no discoverable organic pathology, (4) spastic contractions of the colon. Patients are drawn from referrals to the Digestive Diseases Division of Baltimore City Hospitals. Normal subjects are recruited by notices posted in Baltimore City Hospitals. To assess presence or absence of spasticity in the colon a flexible tube with two 5-cm balloons attached to it is inserted approximately 18 cm into the bowel so that the higher balloon lies in the rectosigmoid area and the second balloon lies in the rectum. This tube is held in place by a hollow metal cylinder which is surrounded by two doughnut-shaped balloons on each side of the anal canal. This system permits us to record contractions of the rectosigmoid and rectum and of the internal and external anal sphincters. The subjects's ability to sense distension of the rectum is assessed by means of a forced-choice discrimination procedure, as follows: The subject is instructed that air will be put into the balloon in only one of two intervals and he should indicate whether it was the first or second time. The order of correct choices is varied in a random sequence. Five ml of air is used to distend the rectosigmoid. The presence of spastic contractions is assessed by adding 20 ml of air to the rectosigmoid balloon every two minutes in a stepwise fashion up to a total of 200 ml so that the colon is gradually stretched. In IBS patients this stretching produces spastic contractions of the colon and often reproduces the type of abdominal pain which patients report. The threshold amount of air (stretch) which produces spastic contractions of the colon remains about the same from one occasion of testing to another unless treatment has been started. Biofeedback treatment involves having the patient watch the polygraph recording and try to inhibit the spastic contractions as the colon is stretched. Instructions to the patient are to try to relax, and to try to learn to sense the contractions as they occur. Practice with visual feedback and verbal encouragement lasts for an hour during each training session. Behavior modification treatment involves training the patient in progressive muscle relaxation, systematic desensitization to reduce the patient's tendency to react to certain situations with anxiety and gastrointestinal symptoms, and a bowel training program to establish regular bowel habits. Normal subjects are seen for a single session in which they are evaluated for ability to perceive rectal distension and for the presence of spastic contractions in the rectosigmoid colon. IBS patients are seen for a total of 22 weeks including a 6-week baseline period and two 8-week treatment periods. Throughout this period they are asked to keep daily records of the frequency and severity of abdominal pain and the frequency of bowel movements. The 6-week baseline begins with a test of sensation and colonic spasticity and ends with a second test for spasticity.



Tests for spasticity are also done at the end of each treatment period. During each treatment period patients are seen weekly for biofeedback training or behavior modification. Half of the patients are randomly assigned to have biofeedback before behavior modification, whereas the other half receive behavior modification treatment first. IBS patients are asked to answer a standard psychological test, the Hopkins Symptom Check List -90, at the start of baseline.

G. There are several criteria which jointly serve to differentiate between a voluntary response and a reflex although none of them is definitive by itself. They are: (1) Stimulus specificity. In accordance with the classical observations of Sherrington any reflex arc has a low threshold for elicitation by one class of stimuli, and a high threshold for elicitation by other classes of stimuli. Related criteria which have been added by subsequent investigators are: (2) that the ability of a stimulus to elicit a reflex is not dependent on learning, whereas the ability of a stimulus to lead to a voluntary response is dependent on prior learning, and (3) voluntary responses may be produced on demand by a conscious and motivated subject in response to verbal instructions. Another criterion for spinal reflexes is (4) that reflexes should be elicitable in decerebrated animals (after a period of recovery from shock), but voluntary responses should not be possible for the decerebrated animal. Fifteen chronically constipated patients (average age 32.5 years) and 7 asymptomatic normals (average age 27.7 years) were compared as follows: A hollow metal tube was inserted into the anal canal. Attached to this tube were two doughnut-shaped balloons which held the tube in place when inflated with 10 ml of air each, and which allowed contraction and relaxation of the external and internal anal sphincters to be detected by attached pressure transducers. A third balloon on the end of a smaller tube was inserted through the hollow tube and into the rectum. Varying amounts of air were injected into the rectal balloon to stimulate the rectum.

H. Test of the hypothesis that the ANS of the aged is functioning differently than that of young adults necessitates age comparisons on the interrelationships among ANS response measures. Comparison of age groups on the correlations between ANS response measures is inadequate because the ANS of young adults does not show a uniform response, and correlations among ANS response measure across individuals often are non-significant. The pattern of intra-individual ANS response thus needs to be investigated and the concept of individual specificity is a means by which to do this. Individual specificity refers to the tendency of an individual to emit the same hierarchy of responses to all stimuli. For example, hypertensives have been reported to have a greater tendency than normotensives to respond to all stimuli with a pressor response. Comparisons of age groups on individual specificity reflects age differences in the adequacy of ANS function because if an individual's psychophysiological response pattern is consistent across stimuli, his response pattern often will be inappropriate to the demands of the situation. Ten young subjects in each decade from 20 to 80 years are being tested. Each subject receives the following stimuli which are present in counterbalanced order for 30 seconds: (1) cold pressor,

(2) mental arithmetic, (3) time estimation, (4) incongruous slides, and (5) exercise. These stimuli were selected because they are effective (i.e., elicit responses) and diverse, the appropriate stimuli by which to assess specificity. Subject responses are breathing rate, skin potential, digital blood flow, heart rate, and blood pressure. Intra-class correlations are used to assess individual specificity. Since health status data are collected as a part of the Baltimore Longitudinal Study, it will be possible to determine if individual specificity is related to disease and/or is predictive of disease.

I. The most widely accepted model of electrodermal function was proposed by Edelberg. This model specifies that the skin comprises two generators, a larger voltage source in the sweat gland and a smaller generator in the epidermis and two resistors. The sweat gland resistor and battery are in parallel with the epidermal resistor and its generator. In experiment I age differences in skin potential level (SPL) were evaluated by varying epidermal resistance through the use of different electrolyte media, and the use of different durations of presoaking the skin in distilled water. Twelve old (60-85) and 12 young (20-30) men were tested at rest and in response to two reaction time tasks. Experiment II was designed to evaluate age differences in the epidermal battery which is thought to be due to the potential difference between the electrolyte and epidermal potassium. This potential is the minimum voltage recorded during resting conditions when the sweat glands are quiescent. It is called basal skin potential level (BSPL). Twelve young and 12 old men were tested. Experiment III was designed to identify the recording conditions under which BSPL would be most stable. Ten young adult men were administered BSPL procedures on two occasions using 4 levels of epidermal hydration. Experiment IV looked at the relationship of health to SPL. The sweat gland battery is thought to reflect sweat gland sodium reabsorption. Therefore, drugs or diseases that influence sodium reabsorption should influence SPL when it is primarily reflecting the sweat gland battery. Skin potential level of nine hypertensives on diuretic therapy and eight untreated hypertensives were compared during BSPL procedures using electrolytes and conditions that varied epidermal resistance.

### Major Findings

A. Approximately 30 patients have completed phases 1 and 2 and are now in phases 3 or 4. About another 30 patients are now in phase 1 or 2. It is much too soon to report any of the results except to note that almost all patients tested to date are able to show acute drops in blood pressure in conjunction with the relaxation or feedback procedures, and a few patients seem to be showing sustained drops in blood pressure over the first three months of treatment.

B. All patients were able to learn to lower their heart rates in the laboratory. In addition four of the six patients showed improved performance on their final (post-training) exercise stress test. The change in performance on the exercise stress test for the four successful patients

ranged from 3% to 113% (time on the treadmill), and from 42% to 461% (treadmill work). Overall means for the six patients were: Time (37%, 2.05 min); Work (108%, 1442 kg.m/min). Five of the six patients showed an increase of the ratio of work done on the treadmill to the product of heart rate and systolic blood pressure (an index of cardiac work).

C. All patients were highly compliant by a number of criteria: (1) All remained in the study until they had completed their programs; (2) Each kept extensive activity records (ranging from 5.8/day to 16.7/day); (3) Pulse rates before and after each listed activity were almost always taken (individual compliance rates ranged from 86 to 100 per cent); (4) There were very few deviations from the hierarchy as determined during the weekly interviews; (5) Patients were very reliable in keeping their appointments with us and with their physicians. Several other observations are noteworthy: a) Two of the patients' wives accompanied their husbands regularly at weekly visits; two others came occasionally. Usually they corroborated their husbands self-reports. Occasionally they added information about symptoms; b) Only one patient complained that pulse taking was inconvenient. All patients, at one time or another, reported that the pulse taking was gratifying because it enabled them to evaluate their recovery in terms which were readily understandable to them; c) As patients improved, there was a decline in pulse rate taking. However, most patients continued to register pulse rates from activity levels that they had already achieved: This behavior was never discouraged; d) All patients were highly reliable in the degree to which they adhered to their hierarchies. However, there were both omission failures and commission failures. One patient failed to participate in a walking program as much as he had contracted, and he prematurely engaged in two activities that he had not agreed upon. Two other patients engaged in moderately strenuous activities on one occasion each. Most patients recorded the deviations from their hierarchies on their worksheets, and they were eager to discuss these behaviors at their weekly visits.

D. The AMA patients differed significantly from the control patients in a number of ways: a) They had less evidence of acute cardiac illness; b) They had more self-reports of alcohol abuse and of emotional disturbances; c) They manifested more behavior problems (as reported by the nursing staff or the house staff) while in the hospital; d) They had fewer deaths in the post-hospitalization period.

E. Although progress on this project has been hampered because of lack of availability of sufficient patients and of the limited availability of urological co-investigators, pilot results with three patients indicate that it is possible for incontinent patients to modulate bladder and sphincter performance. Because of the few patients studied follow-up results are inconclusive. Nevertheless, the data suggest that these patients may be able to sustain some degree of improved urinary function.

F. This project is still in progress. No findings can be reported at this time.



G. In response to rectal distension with 50 ml and 40 ml of air, only half of constipated patients showed a phasic external anal sphincter contraction, and one of 7 normals did not show a contraction to 40 ml. Moreover, the strength of phasic external anal sphincter contractions were significantly smaller on the average in constipated patients compared to normals. Differences between constipated patients and normals were not attributable to loss of sensation in the constipated patients because: (1) all patients could perceive at least 20 ml distensions of the rectum; (2) sensory threshold was unrelated to whether the patient or normal showed an external anal sphincter contraction in response to rectal distension; and (3) all patients and subjects showed reflex relaxation of the internal anal sphincter to rectal distension even when they showed no external sphincter contraction. We tested the effects of instructions to not contract in one normal subject who showed contractions of the external anal sphincter with stimuli of 50 ml and 40 ml. This subject was immediately able to sustain rectal distension with 50 ml and 40 ml without contracting the external anal sphincter. These observations show that the phasic external anal sphincter contraction following rectal distension is a voluntary response and not a reflex.

H. This study has just begun. There are no findings at this time.

I. Experiment I - Age differences in SPL occurred only with high epidermal resistance with aged having lower SPL than young adults. These results suggest that the aged may have a lower sweat gland potential than young adults. This age difference is observed only when epidermal resistance is high because young adults have a lower ratio of epidermal resistance to sweat gland resistance than the aged. Thus, the sweat gland potential will contribute less to the recorded skin potential level of young adults unless their epidermal resistance is increased. These results also may reflect an age difference in the epidermal potential. Experiment II - There were no age differences in BSPL for either high epidermal resistance or low epidermal resistance conditions. However, when sweat gland activity occurred the aged had a significantly lower SPL when high epidermal resistance conditions were present. These results support the hypothesis that there are age differences in sweat gland potential, and that to observe this age difference epidermal resistance must be high. Experiment III - Epidermal hydration influences the intraindividual stability of BSPL over time. Hydration increases BSPL stability with a 30 min. pretreatment of soaking in distilled water producing a repeatable BSPL. The mechanism of this effect seems to be due to a hydration induced reduction in the sweat gland contribution to SPL. Experiment IV - There were no differences in mean blood pressure between the hypertensive patients on diuretic therapy and the untreated hypertensives. The patients receiving diuretics had significantly higher SPL than the untreated hypertensives for both BSPL, and SPL when sweat gland activity predominates. This difference occurred for both conditions of epidermal resistance although the differences were larger with conditions of high epidermal resistance. These results suggest that hypertensives on diuretics and untreated hypertensives differ both in terms of the sweat gland battery and the epidermal battery.



Significance to Bio-medical Research and the Program of the Institute:

This laboratory is carrying out one of the major programs in the world in the rapidly developing field of Behavioral Medicine, the application of behavioral techniques and principles to the analysis of patho-physiological process and to the treatment of medical patients. The primary focus of research in this laboratory is to improve the care and treatment of patients suffering from disorders which often occur in the middle and late years of adult life. The projects in this program range widely and include studies of the management of behavior which is potentially harmful to the patient; studies which attempt to use behavioral strategies to modify aberrant physiological responses; and studies which attempt to use reproducible behavioral procedures to characterize physiological mechanisms.

Proposed Course of Project:

A. This project will continue until approximately 100 patients complete all phases of the study.

B. The present study had two major shortcomings: 1) the lack of control condition precluded any separation of the treatment effect from the effect of repeated testing; and 2) the possible inadequacy of the procedure used to optimize transfer from the laboratory training condition to the exercise stress test condition. A study about to be implemented will compensate for these shortcomings by: 1) adding a delayed treatment group to assess the effect of repeated testing; and 2) training patients to control heart rate during conditions of moderate exercise.

C and D. Further studies designed to evaluate treatment non-compliance and to treat such behavior will be implemented. The development of a cardiac rehabilitation program at Baltimore City Hospital will enable us to study patients in such a program. Examples of projects which may be implemented include programs to control smoking behavior in post-infarction patients, programs to develop means of predicting which patients are at high risk for non-compliance and programs for evaluating the behavioral complications of angina pectoris.

E. The emergence of a urology service at Baltimore City Hospital, and the possible establishment of an adult incontinence clinic should permit us to study an adequate number of patients with adequate, continuing urological support.

F. Data collection will continue.

G. This project is completed and a manuscript is in preparation.

H. Data collection will continue. Longitudinal Studies--i.e., repeat testing of the subjects--is projected at two years and ten years.

I. Research will focus on the conditions that will enable skin potential level to better reflect the sweat gland potential and produce an intra-individually stable measurement. Pilot data suggest that lowering the concentration of the high resistance electrolyte provides an even better estimate of the sweat gland potential. Once an intraindividually stable sweat gland potential measure has been obtained, the diuretic effect will be systematically investigated in a longitudinal study. Hypertensive patients will be tested before being placed on diuretic therapy and during diuretic therapy.

Publications:

Baile, W. F., Engel, B. T.: A behavioral strategy for promoting treatment compliance following myocardial infarction (MI). Psychosom. Med., in press.

Garwood, M. K., Engel, B. T., and Quilter, R. E.: Age differences in the effect of epidermal hydration on electrodermal activity. Psychophysiology, in press.

Engel, B. T.: The treatment of fecal incontinence by operant conditioning. Automedica. 2: 101-108, 1978.

Engel, B. T.: Behavioral applications in the treatment of patients with cardiovascular disorders. In Basmajian, J. V. (Ed.) : Biofeedback. Baltimore, Waverly Press, Inc., 1978, pp. 166-175.

McCroskery, J. H., and Engel, B. T.: Biofeedback and emotional behavior. In Christie, M., and Mellett, P. (Eds.): Psychosomatic Approaches in Medicine, Volume One: Behavioral Approaches. Chichester, England, John Wiley & Sons, Limited, 1978.

Rubin, S. A., Quilter, R., and Battagin, R.: An accurate and rapid inflation device for pneumatic cuffs. Am. J. Physiol: Heart Circ. Physiol., in press.

Whitehead, W. E., Aleo, S., Edwards, T. L., Jr., Engel, B. T., Gregory, C., Latimer, P., and Schuster, M. M.: Gastrointestinal biofeedback: Report of the task force to the Biofeedback Society of America. Biofeedback Self-Reg., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AC 00068-16 LBS
PERIOD COVERED <p style="text-align: center;">October 1, 1977 to September 30, 1978</p>		
TITLE OF PROJECT (80 characters or less)  <p style="text-align: center;">Operant Performance, Memory and Aging</p>		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <div style="display: flex; justify-content: space-between;"> <div>           PI: Charles L. Goodrick            OTHERS: None         </div> <div>           Research Psychologist            None         </div> <div>           LBS GRC NIA         </div> </div>		
COOPERATING UNITS (if any) <p style="text-align: center;">Baltimore City Hospitals</p>		
LAB/BRANCH <p style="text-align: center;">Gerontology Research Center - Laboratory of Behavioral Sciences</p>		
SECTION <p style="text-align: center;">Learning &amp; Problem Solving</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NIA, NIH, Baltimore, Md. 21224</p>		
TOTAL MANYEARS: <p style="text-align: center;">.20</p>	PROFESSIONAL: <p style="text-align: center;">.10</p>	OTHER: <p style="text-align: center;">.10</p>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-around;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS         </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES         </div> <div> <input checked="" type="checkbox"/> (c) NEITHER         </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 5px;"> <div> <input type="checkbox"/> (a1) MINORS         </div> <div> <input type="checkbox"/> (a2) INTERVIEWS         </div> </div>		
SUMMARY OF WORK (200 words or less - underline keywords) <p>             The major purpose of this project is to analyze <u>complex maze learning</u> of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in <u>operant performance</u> for young and aged <u>rats</u> or <u>mice</u>, and to determine factors which may act to improve performance, and also improve <u>retention</u> of the learned responses.           </p>		

GRC/LBS-155

Project Descriptions:

Objectives: The objectives of one phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals. The general objectives of the other phase of this project are: (1) to analyze complex maze learning of young and aged animals; and (2) to determine variables which may act to enhance or retard maze learning ability.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6 units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and retroactive.

Major Findings: When rats were given a choice between bar pressing for food pellets or eating pellets from a dish within an operant test chamber ("free loading"), research workers in another laboratory found that just one rat out of 200 obtained all of the food pellets from the food dish during the single 40-minute test period. Eighty-eight out of 200 rats earned more than half of all the food pellets eaten during the test period by bar pressing. These results were interpreted as showing a preference for a more effortful response over a less effortful response to obtain the food reward, a result which contradicts a large body of psychological research. Since this experiment was published, other research studies have replicated these data.

This experiment was designed to examine in greater detail the free-loading phenomenon. After operant bar press training on continuous reward in a two-bar test chamber, with one reward bar and one neutral bar, rats (N = 45) were divided into three groups and given eight 15-minute bar pressing trials under the following conditions. One group (G1) continued bar press training with the milk-sucrose available as a reward and also freely available in the test chamber. A second group (G2) was given extinction trials with milk-sucrose freely available, but not available by bar pressing, and a third group (G3) was given extinction trials with milk-sucrose solution not available. G1 increased in reward-bar pressing performance over trials, then decreased to a low level. G1 and G3 did not differ significantly in



reward-bar responses, but G1 increased reward-bar responses within trials, while G3 decreased reward-bar responses within trials. G1 did not decline in percentage of reward-bar responses over trials, while G2 and G3 did. The results were consistent with the hypothesis that hungry rats prefer the less effortful of two alternatives for food, and that patterns of bar pressing extinction and performance are changed by allowing access to free food during extinction or continued training. In addition, the two-bar operant test was shown to be a reliable and valid procedure for the study of performance and learned components of experimental extinction, and may be very useful in the study of differing age groups.

Significance to Bio-Medical Research and the Program of the Institute:

Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to: (a) time contingent vs. response contingent partial reinforcement; and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We will also initiate preliminary studies of perceptual learning in humans to determine the generality of the massed practice effects found for aged rats.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00069-13 LBS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Exercise, General Activity Level and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Teena M. Wax	Postdoctoral Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center LBS

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Md. 21224

TOTAL MANYEARS:

.80

PROFESSIONAL:

.40

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is to determine the effect of voluntary wheel exercise upon behavior for animals tested in lifespan developmental research, and in addition to consider the effect of voluntary wheel exercise upon longevity. Another goal of this research is to increase the period of vigorous activity during later stages in the lifespan of the rat or mouse.

GRC/LBS-158

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span; (2) to examine behavioral and longevity differences among animals which differ in physical activity level; and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise. Hungry animals also may be rewarded with food for running. A technique to control experimentally body weight and to increase voluntary exercise utilizes feeding of animals on a periodic schedule, such as every other day. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions (See Project Z01 AG 00061-15 LBS Behavioral genetics and aging).

Major Findings:

A. An investigation of voluntary wheel exercise and life span of Wistar rats been completed. In this study, paired male and paired female rats (N = 140) were maintained in cages with attached activity wheels (experimental groups) or in cages without access to activity wheels (control groups). The 40 males (Group 1, N = 12; Group 2, N = 28) and 40 females (Group 1, N = 12; Group 2, N = 28) allowed access to activity wheels were started under these conditions when 45 days old. The two experimental groups were started six months apart; Group 1 rats were started in July, 1973, and Group 2 rats in January 1974. Control pairs of 28 males and 32 females were 45 days old at the same time as experimental Group 1 male and female rats. The mean life span of the male control group was 20.7 months, while the mean life span of the male experimental animals was 24.7 months. The difference in life span for the male experimental group versus the control group was highly statistically significant,  $t_{(66)} = 4.28$ ,  $p < .001$ . The mean life span of the female control group was 26.2 months, with the mean life span of the female experimental animals 29.2 months, also a statistically significant difference,  $t_{(70)} = 2.52$ ,  $p < .01$ . These differences provide strong evidence that voluntary exercise significantly increases life span.

Additional analyses have now been completed concerning the relations of body weight increment, peak body weight and longevity. Greater duration of body weight increment resulted in a greater life span both within and between groups. Within groups, high body weight was also positively correlated with longevity. Both of these results are consistent with previous published work with mice in this laboratory. Rats allowed voluntary exercise did not show the normal age related decrement in metabolic rate obtained for rats maintained in cages and not allowed voluntary exercise. The finding that groups high in mean metabolic rate were high in mean longevity contradicts a major gerontological hypothesis that metabolic rate is negatively related to life span.

The mean growth duration was significantly greater for rats allowed voluntary wheel exercise than for controls,  $F_{(1,136)} = 8.59$ ,  $p < .001$ , and female

rats had a significantly longer mean growth duration than male rats,  $F(1,136) = 38.68$ ,  $p < .001$ , Table 1. Mean peak body weight was higher for control rats than for rats allowed voluntary wheel exercise,  $F(1,136) = 25.55$ ,  $p < .001$ , and male rats were significantly higher in mean peak body weight than female rats,  $F(1,136) = 94.58$ ,  $p < .001$ , Table 1. Interactions of sex and caging condition were not significant for growth duration and peak body weight.

Correlations of longevity and growth rate (peak body weight divided by month of peak body weight) were all statistically significant, ranging from  $-.43$  to  $-.57$ , (see Table 2); the slower the growth rate, the longer the life span. The correlations between longevity and growth duration were all statistically significant (Table 2) ranging from  $+.47$  to  $+.74$ . Although all correlations between longevity and peak body weight were positive (Table 2), they were statistically significant only for the male groups. The higher the peak body weight, the greater the longevity of male rats.

The mean body weights, number of rats and standard errors are given for each group from 1.5 to 27 months of age in Table 3. At the start of the study, weight differences were not different when control rats were compared with rats allowed voluntary wheel exercise. However, mean body weights were significantly greater for control rats than for rats allowed voluntary wheel exercise at every age after the start of the study (2.5 through 24.0 months), except for the last age (27.0 months.).

A repeated-measures analysis based on body weight measures obtained highly significant effects, with mean body weight of rats allowed voluntary wheel exercise lower than mean body weight of control rats,  $F(1,44) = 68.00$ ,  $p < .001$ , and mean body weight of female rats lower than mean body weight of male rats,  $F(1,44) = 438.54$ ,  $p < .001$ . The second order interaction of caging condition, sex, and age was also significant,  $F(6,264) = 16.64$ ,  $p < .001$ . Body weight differences between control rats and rats allowed voluntary wheel exercise were greatest for male rats at the middle portion of the life span (9, 12 and 15 months), but were greatest for female rats later in the life span (15, 18, and 21 months).

The correlations of ultimate longevity and body weight at a specific age were at 1.5, 2.5, 3.5, 4.5, 6.0, 9.0, and 12.0 months of age. Although 20 of 28 of these correlations were negative, 10 of these were less than  $-.20$ , too low to have predictive significance.

Mean metabolic rates of all rats tested are given in Table 4. For both male and female groups, the mean metabolic rates of rats allowed voluntary exercise was significantly higher than the metabolic rate of control rats at all ages from 12 months to 21 months. At the earliest age, 3 months, differences were not statistically significant. A repeated measures analysis of variance using 12 of the same subjects in each group was also computed. Female rats had higher mean metabolic rates than male rats,  $F(1,44) = 353.52$ ,  $p < .001$ . Also, rats allowed voluntary wheel exercise had higher mean metabolic rates than control rats,  $F(1,44) = 34.81$ ,  $p < .001$ .



and mean metabolic rate decreased with increasing age,  $F(6,264) = 5.98$ ,  $p < .01$ . The only significant interaction was that of caging condition by age. Metabolic rate remained at a stable level with increasing age for rats allowed voluntary wheel exercise, but metabolic rate decreased with increasing age for rats not allowed voluntary wheel exercise.

B. In an attempt to increase voluntary wheel exercise later in the life span, additional pairs of male rats either have been placed in wheels or placed as controls in normal cages, with food being restricted to every other day (EOD). Two groups of 12 experimental rats ( $N = 24$ ) and two groups of 12 control rats ( $N = 24$ ) are now being tested. The preliminary results are promising. The oldest groups (12 experimental and 12 control rats) are now 33 months old. Eleven of those twenty-four are still alive and voluntary exercise is still considerably above that obtained for old rats fed ad libitum. The results of this study will yield much basic information. (1) There will be available a clearly defined technique to increase dramatically the life span of the GRC male rat. (2) The rats which are allowed voluntary wheel exercise are similar in body weight to rats not allowed voluntary wheel exercise (both EOD fed groups). Therefore, the effects of voluntary wheel exercise may be clearly determined independently of body weight. (3) This EOD feeding procedure has yielded a much increased level of voluntary wheel exercise in advanced old age, compared with rats fed ad libitum. The entire results will be available when all the rats have died approximately 12 to 24 months from now.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) may be increased experimentally for old and senescent animals. It is also important to examine the role of heredity with respect to voluntary exercise throughout the entire lifespan and the effect of exercise upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in the development of methods which can increase the productive later years of aged humans.

Proposed course of the project: The rat studies of wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats and to determine the amount of voluntary exercise during advanced old age. Additional studies will determine the effect of reducing food intake by restricted, every other day feeding upon voluntary wheel exercise and longevity. Also, more control groups will be added to obtain continuing data with respect to normal longevity.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for

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young and aged mice which are allowed voluntary control of lighting conditions within the home environment. Studies of the effect of dietary protein changes late in the life span upon voluntary wheel exercise and longevity for rats will continue.

Publication: None

Table 1

Means and Standard Errors of Longevity, Growth Rate, Growth Duration, and Peak Body Weight for

Male and Female Rats as a Function of Voluntary Wheel Exercise

Male	N	Longevity (mo.)	Growth Rate <sup>a</sup>	Growth Duration (mo.)	Peak Body Weight (gm)
Control	28	20.7 ± .6	51.2 ± 1.4	13.5 ± .4	677.3 ± 9.2
Exercise	40	24.7 ± .7	35.4 ± 1.1	16.1 ± .4	555.5 ± 6.3
Female					
Control	32	26.2 ± .8	24.6 ± .9	19.3 ± .5	463.6 ± 8.6
Exercise	40	29.2 ± .8	17.6 ± .6	22.4 ± .6	386.5 ± 2.0

Note: <sup>a</sup> (Peak body weight divided by month of peak body weight)

Table 2

## Correlations of Longevity with Growth Rate, Growth Duration, and

## Peak Weight, for Each of Four Groups of Rats

Male	Longevity X Growth Rate	Longevity X Growth Duration	Longevity X Peak Weight
Control	- .43 <sup>x</sup>	+ .51 <sup>*</sup>	+ .39 <sup>x</sup>
Exercise	- .57 <sup>*</sup>	+ .47 <sup>*</sup>	+ .73 <sup>*</sup>
Female			
Control	- .54 <sup>*</sup>	+ .74 <sup>*</sup>	+ .11
Exercise	- .46 <sup>*</sup>	+ .60 <sup>*</sup>	+ .06

\*  $\underline{p} < .01$ X  $\underline{p} < .05$



Table 3

Number of Rats, Mean Body Weights, and Standard Errors as a Function of Voluntary

Wheel Exercise for Male and Female Wistar Rats

		Age (Months)					
Male		1.5	2.5	3.5	4.5	6.0	9.0
	N	28	28	28	28	28	28
Control	M	182.0	365.2*	446.8*	499.1*	535.1*	641.9*
	SE	3.0	4.7	5.7	6.7	8.0	6.9
	N	40	40	40	40	40	40
Exercise	M	177.7	329.1	373.4	424.5	447.7	514.4
	SE	3.7	4.7	5.0	5.6	6.1	7.1
Female							
	N	32	32	32	32	32	32
Control	M	145.8	226.4*	261.9*	278.2*	301.3*	365.7*
	SE	1.5	2.2	2.6	8.7	4.8	5.6
	N	40	40	40	40	40	40
Exercise	M	146.5	210.8	238.3	259.0	271.1	296.8
	SE	1.9	2.2	2.5	2.6	2.8	3.8

Table 3 Cont'd.

Number of Rats, Mean Body Weights, and Standard Errors as a Function of Voluntary  
Wheel Exercise for Male and Female Wistar Rats

		Age (Months)					
		12.0	15.0	18.0	21.0	24.0	27.0
Male	N	28	28	25	15	2	1
	M	660.6*	658.5*	615.2*	553.2*	552.0*	475.0
	SE	7.8	12.8	16.7	24.3	8.5	-----
	N	40	38	38	34	23	10
Exercise	M	534.1	547.9	538.3	514.2	489.7	477.0
	SE	7.3	6.7	6.5	6.7	9.2	9.6
Female	N	32	32	32	23	18	15
	M	365.7*	426.1*	443.4*	449.6*	428.4*	404.0
	SE	6.2	10.0	9.5	10.8	13.0	18.2
	N	40	39	38	36	34	24
Exercise	M	313.5	328.7	343.4	362.0	367.0	384.7
	SE	3.8	4.5	5.6	5.7	6.2	24.6

\*  $p < .01$  Control vs. Exercise

Table 4

Number of Rats, Mean Metabolic Rate (ml O<sub>2</sub>/gm/hr X 100), and Standard Errors  
as a Function of Voluntary Wheel Exercise for Male and Female Wistar Rats

Male		Age (Months)							
		3	6	9	12	15	18	21	24
Control	N	12	12	12	12	12	12	12	----
	M	99.2	91.5*	88.2*	82.3*	77.8*	78.4*	74.2*	----
	SE	4.1	3.6	2.2	2.2	1.7	3.0	1.8	----
Exercise	N	12	40	40	40	38	38	34	20
	M	94.8	100.9	95.3	99.3	97.3	97.8	100.4	96.4
	SE	4.8	1.5	1.7	1.8	2.2	2.2	1.7	4.8
Female									
Control	N	12	12	12	12	12	12	23	16
	M	121.4	116.8*	119.9	110.1*	107.6*	107.7*	101.1*	103.4*
	SE	6.4	2.8	4.1	2.0	2.4	2.4	2.5	3.2
Exercise	N	12	40	40	40	39	38	36	30
	M	115.7	124.1	124.0	118.1	127.4	119.7	118.2	118.7
	SE	3.8	1.9	2.6	1.6	2.7	2.4	1.8	3.2

\* P < .01, Control vs. Exercise

NIA Annual Report  
October 1, 1977 through September 30, 1978  
Gerontology Research Center  
Laboratory of Cellular and Molecular Biology

The Laboratory of Cellular and Molecular Biology is a newly created laboratory. The purpose of the reorganization of the Gerontology Research Center which resulted in the formation of this laboratory was to bring together various research projects which are related through a common interest in the genetic basis for the biological aging process. The Section on Inorganic Biochemistry is concerned with fundamental studies at the molecular level of biological macromolecules involved in genetic information transfer. Age changes are investigated in systems involving these molecules, and particular emphasis is placed on the beneficial, as well as harmful, effects of metal ions that interact with these molecules. The Section on Macromolecules is concerned with the interaction of cells with synthetic macromolecules. Age changes on the cell surface are probed with specially designed macromolecules, and potential polymeric drugs are synthesized. Physico-chemical alterations of chromatin and its transcription are monitored, and age changes in gene expression and in abnormal protein degradation are studied. The Section on Cellular Aging and Genetics is concerned with cell replication and repair of DNA damage as a function of aging. It also deals with the mechanism of parental age effects.

Recent studies on the structure of chromatin indicate that it consists of DNA bound tightly to a combination of H2A, H2B, H3 and H4 histones in so-called nucleosomes, which are bridged by DNA bound by histone H1. The nucleosomes can be pictured as strings on a bead. The chromatin structure can be readily probed by enzymes that hydrolyze DNA, e.g., micrococcal nuclease, which indicate that different portions of the chromatin DNA have different accessibility. Thus the bridging DNA is most readily attacked, leaving the nucleosomes, which can then be further broken down into core and linker regions. We have previously found very significant differences in the early reaction rates of young (8-9 months) and old (22-27 months) chromatin with micrococcal nuclease. Equilibrium experiments, in which the chromatin is treated with various concentrations of enzyme, lead to extents of degradation that are quite similar for young and old chromatin, within the limits of variability in chromatin of the same age. From these results we cannot conclude that any age differences exist in the number of nucleosomes or in the length of the spaces between the nucleosomes. The difference in the early reaction rates, which we have confirmed, thus indicates that the only age change is in the structure of the bridging DNA complex.

Recently, Dr. E. Moudrianakis of Johns Hopkins University has isolated a protease from rat liver chromatin that cleaves histone H2A in a very specific way so that the 15 amino acids on the C-terminal of the protein are removed. In collaborative work with Dr. Moudrianakis, it has been found that the activity of this enzyme is greater in old than in young rat preparations. The biological significance of this enzyme is still unknown, but, from the fact that it serves to modify a histone, one can guess that it may be involved in genetic regulation. However, the results and conclusions with this enzyme are preliminary and subject to further study.



The following age changes have been found in mouse and rat tissues:

(1) Extractability of chromatin proteins decreases with increased age for mouse liver and brain tissues. (2) Ribosomal RNA hybridization efficiency decreases with increased age for mouse liver, brain, kidney, heart and spleen tissues. (3)  $T_3$  hormone binding capacity of chromatin decreases with increased age in liver tissue of rats and remains unchanged in the old 6-month post-hypophysectomized animals.

An observation of potentially great interest in aging research has been the discovery by Dr. D. Crapper of the University of Toronto that the brains of those who have died of Alzheimer's disease contain very high local accumulations of aluminum. Though there is some dispute about these findings in that Wisniewski believes that aluminum accumulates in brain generally with age, it is obvious that this accumulation, whether or not it is related to Alzheimer's disease, could be of importance. The discovery in Dr. Crapper's laboratory that aluminum is associated with chromatin in the cell therefore suggests that the site responsible for the effect of aluminum may be DNA. In our previous studies of metal-DNA interaction we have neglected aluminum and are now making up the deficit through collaboration on this project with Dr. Crapper and S. Karlik of the University of Toronto. Results to date indicate that aluminum does bind to DNA and has dramatic effects on its structure. The results indicate that aluminum forms two types of complexes with DNA, of two different stabilities and structures, but both of these form crosslinks between the DNA strands. It is tempting to suggest that such crosslinks could be responsible for the Alzheimer lesions, but of course much needs to be done to verify such a possibility.

Errors in protein synthesis can result from the presence of too high concentrations of metal ions or from the introduction of antibiotics into the protein synthesizing system. We have shown that the concentrations of metals that produce errors will also cause mispairing of bases, so that the errors may be produced by mistaken identity in codon-anticodon interaction. Higher concentrations of metal ions are required to produce errors in protein synthesis with mammalian ribosomes than with bacterial ribosomes. It appears then that mammalian ribosomes are better made to withstand stress than bacterial ribosomes. From this observation it becomes reasonable to use stress from increasing concentrations of metal ions to test the ability of ribosomes to maintain fidelity of translation. In this way old and young ribosomes can be compared. Stress on the ribosomes by the addition of antibiotics can also be used to detect differences in ribosomal fidelity due to aging. Phenylalanine is correctly incorporated into protein, and mistranslation of one letter on the codon causes leucine incorporation. We therefore carried out double labeled experiments using [ $^{14}\text{C}$ ]Phe-tRNA and [ $^3\text{H}$ ]Leu-tRNA charged with their respective amino acids and determined incorporation rates of these amino acids into polymerized molecules. We prepared ribosomes from the livers of 6-month and 12-month old male rats as well as cytosol protein fractions to be used as a source of enzymes for tRNA acylation and elongation factors. Misincorporation of leucine with rat liver ribosomes was in fact observed, and linear reaction rates could be followed for both Phe and Leu incorporation. Our results so far show that old ribosomes exhibit diminished incorporation rates for both Phe and Leu but that the important ratio of Leu:Phe remains rather similar, of the order of 0.05, for the small number of animals tested so far.

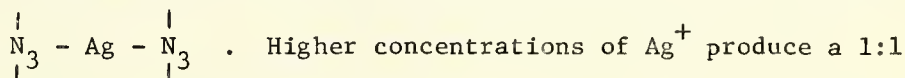
Certain antibiotics can also induce errors in protein synthesis. Challenging the rat liver ribosome-poly(U) system with the antibiotic, paromomycin, led to a ratio Leu:Phe in the range of 0.15. Again, even at this high level of misincorporation, we were unable to obtain consistent age differences.

These results could indicate that fidelity of protein synthesis is not altered by aging, either through codon-anticodon mispairing or through conformational changes in the ribosome. However, it may be that the changes are too subtle for us to have detected.

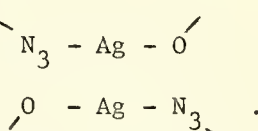
A long-standing concern of this laboratory has been the interactions of metal ions with nucleic acids. These interactions are important in part because of the participation of metal ions in the physiological processes that involve the nucleic acids, and in part because metal ion reactions can be used to probe the structure and function of the biological molecules.

Many of the reactions of metal ions with polynucleotides serve to disorganize ordered structures, but in other cases the metal ions can produce order from disorder. Such is apparently the case with  $\text{Ag}^+$  ions, which produce a high degree of organization by reacting with unordered forms of polyuridylic acid [poly(U)] and polyinosinic acid [poly(I)].

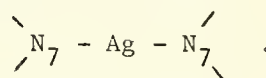
Poly(U) forms two double helical complexes with Ag. A 2:1 complex, poly( $\text{U}_2\text{-Ag}$ ), is formed at low Ag concentration and high pH, and contains the structure



complex formulated as poly ( $\text{U}_2\text{-Ag}_2$ ), with the structure



Poly(I) also forms two complexes with Ag. At low pH a 2:1 complex formulated as poly ( $\text{I}_2\text{-Ag}$ ) is formed, with the structure



At high pH a 1:1 complex formulated as poly ( $\text{I}_4\text{-Ag}_4$ ) is produced with a structure in which each  $\text{Ag}^+$  is bound to  $\text{N}_1$  of one base and  $\text{O}_6$  of another base to form a ring  $(\text{-N-Ag-O-})_4$ . This structure is similar to the 4-stranded poly(I) structure produced by very high salt concentration, except that Ag bonds displace hydrogen bonds.

The proper function of the adult mammalian organism is dependent upon maintaining the correct differentiated state of many types of cells. Our aim is to examine the age-dependent stability of the differentiated cell by determining if improper specific and general gene expression occurs in specific cell populations as a function of age. The method used to investigate improper specific gene expression is to search for the presence of the RNA transcribed by this gene using a radioactive complementary DNA (cDNA) probe. The cDNA probe is obtained by isolation of pure messenger RNA known to code for a specific gene product and then to use this RNA as a template with reverse transcriptase enzyme to synthesize the radioactive

cDNA probe. RNA is then extracted from the nuclei and/or cytoplasmic fractions of certain tissues and the presence of RNA complementary to the cDNA probes determined by DNA-RNA hybridization techniques.  $\alpha$  and  $\beta$  globin RNAs were found in liver and brain tissues of the long-lived young C57BL/6J mouse strain and to increase in relative concentrations with age. A similar change was found for the genes complementary to the c-type virus MuLV cDNA probe. Similar studies were made using the short-lived AKR mouse strain having an unusually high frequency of leukemia. A large age-dependent increase was found for the presence of c-type virus RNA in the thymus gland, but no accompanying expression of the globin genes in either the thymus, brain or liver tissue was found. The expression of globin genes in neurons and adult liver tissue, plus the increase of this expression in older animals, is clearly an unexpected finding and supports the hypothesis that the ability of cells to maintain a differentiated state does decrease with increasing age. Moreover, the age-dependent derepression found for the c-type viruses, which have been indicated as a causative factor in leukemia, suggests that a general decrease in the ability of cells to maintain the repressed state of many other endogenous virus genes may be occurring with increasing age.

Selective degradation of altered protein has been determined in vivo by measuring the relative rate of degradation of proteins which have incorporated normal vs. amino acid analogues. For this study, we used the wild-type rodent species Mus with a lifespan potential of three years and Peromyscus with a lifespan potential of about eight years. The major analogue used was canavanine, an analogue of arginine. Briefly, the technique is to first inject  $^3\text{H}$ -arginine intraperitoneally into a mouse and after about 2 hours to inject  $^{14}\text{C}$ -canavanine. The relative rate of degradation of the two populations of labeled proteins are then determined by measuring the  $^3\text{H}/^{14}\text{C}$  ratio of acid precipitated proteins as a function of time after injection. The longer-lived rodent species, Peromyscus, appears to have about a two-fold greater capacity to discriminate and degrade the abnormal protein. It is possible, however, that these results might be explained by the degradation rate being a function of the relative amount of amino acid analogues incorporated into the protein. This possibility is currently being investigated.

Superoxide dismutase levels were measured in crude cytoplasmic extractions using a standard assay method. For this study, five different primate species, ranging in lifespan potential from 20 to 100 years, were used. Superoxide dismutase activity was found to increase in brain tissue with increased lifespan potential of the primate species so far tested by a factor of about three-fold. An inverse function in the level of guanylate cyclase was found in the same species. These studies support the concept that an increase in the level of a common set of repair and protective processes in the mammalian species may play an important role in governing their different aging rates.

The ability of replicating cells to respond to DNA damage both in vitro and in vivo has been investigated as a function of age. For measurement of cell replication in vivo, young and old animals were infused intravenously with BrdU. At increasing time intervals, these animals were sacrificed and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by



characteristic banding patterns. By utilizing the BrdU-differential staining technique both in vitro and in vivo, the frequency of sister chromatid exchanges (SCE) has been measured. This frequency reflects the response of cells to DNA damage.

Utilizing the BrdU-differential staining techniques, we have developed a new approach to measuring cell replication in vivo as well as in vitro. The technique is rapid, reproducible, capable of determining cell cycle times and can be performed under conditions where cellular replication can be demonstrated to not be affected by the procedure. With this new technique, studies of human lymphocyte cultures in vitro and rat and mouse bone marrow cells in vivo have revealed a clear decrease in cell replication as a function of aging.

Analyses of SCE frequencies have demonstrated a high statistically significant decline in these events in vitro in human skin fibroblast cultures derived from old donors (compared with cells from young donors) and in late passage IMR-90 cells (when compared with early passage cells, "in vitro aging"). Examination of bone marrow cells from C57BL/6J mice and Wistar rats, where studies were conducted entirely in vivo, also revealed a significant decline in the frequency of mitomycin C (MMC) induced SCEs. To demonstrate that these findings were not due to a specific response to MMC, similar results were obtained in vitro with EMS and AAF, two potent alkylating agents, and in vivo with cyclophosphamide and adriamycin, an intercalating agent. Examination of the kinetics for this decline in mutagen-induced SCE indicates that induced SCE frequencies remain stable during early adulthood (6-18 months in mice and rats) and then decline with further aging (19+ months).

Examination of MMC, cyclophosphamide and adriamycin-induced SCE in AKR mice, which display a predisposition toward malignancy and early mortality, revealed a similar decline in SCE frequencies. This decrease in mutagen-induced SCE frequencies is also present in AKR embryonic cells in vitro. F<sub>1</sub> hybrids of AKR and C57BL/6J mice have intermediate frequencies of mutagen-induced SCEs.

Studies of SCE induction by a number of compounds in vivo indicate that our system is a sensitive measure of in vivo mutagen and carcinogen exposure.

Further studies with skin fibroblast cultures derived from young and old human donors indicate that neither insulin nor EGF receptors change as a function of aging and that macromolecular synthesis is unaltered in old cells.

The application of the BrdU-differential staining technique to cell kinetic measurements has provided a new and sensitive tool to examine cell proliferation both in vitro in cultured human cells and in vivo in intact animals. These studies have already shed light on the controversy over whether cell proliferation is diminished with in vivo aging. Our results indicate a consistent decline in cell replication both in vivo and in vitro. Measurements of SCE frequencies were shown to be a sensitive indicator of DNA damage both in vivo and in vitro. Our studies in four



separate systems all indicate an altered response of old cells to induced DNA damage. Since SCE appear to be closely related to chromosomal structure, these results suggest a significant alteration in chromosomal structure with cellular aging. The finding of impaired SCE formation in AKR mice at an early age indicates that alterations in chromosomal structure related to SCE may play a role in the development of malignancy which characterizes this mouse strain and leads to its early death. This mouse strain may thus be a good model system for aging research.

An effort to design macromolecular drugs that can react at the cell surface is based on the fact that macromolecules have only limited potential to penetrate into the inside of cells; thus they represent suitable reagents for the cell surfaces. The cellular surface is an ideal location for drug action; if a suitable macromolecular and cell selective drug can be obtained, a pharmacological intervention can be made without burdening the interior of any cell organism with foreign compounds. In these early stages the objective is to obtain biologically active macromolecules and correlate their activity with their structure. Methods for attachment of small molecular weight compounds to polysaccharides were investigated and the stability of the attachment measured under physiological conditions. These compounds were then tested on (a) mouse erythroleukemic cells which grow in suspension and thus are easily obtainable in bulk, (b) human fibroblast cells, which grow in monolayers and are thus obtainable only in smaller amounts, and (c) human lymphocytes.

Nucleic acids in the presence of carcinogens are strongly adsorbed to the surface of cells in vitro. Using polyuridyate and proflavine, it was shown that the adsorption occurs on the anionic polysaccharides on the cell surfaces and that the amount of these surface components may be measured in that way. Investigation of a large variety of normal and genetically defective human cells from young and old donors and the same cells transformed by SV40 indicated that all these cells have the same amount of surface anionic polysaccharides when compared at the same cell density. Human fibroblast aged in vitro had, compared to all the other cells, higher amounts of surface receptors.

Residues of Triton X-100 were attached to the polysaccharide inulin. The resulting detergent was effective in the solubilization of proteins and phospholipids from membranes of human erythrocytes and led to a release of reverse transcriptase activity from the membrane enveloped virions of murine leukemia. The Triton X-100 inulin derivative inactivated  $\beta$ -adrenergic receptors from frog erythrocytes in a dose related manner similar to the inactivation produced by Triton X-100. On the other hand, digitonin, a detergent containing a bulkier hydrophobic group, did not cause inactivation. On the basis of its Triton X-100 content the inulin derivative was found to be less detrimental to the growth of murine erythroleukemic cells in vitro than Triton X-100 alone when short (2-4 hour) exposure was used, but this difference disappeared at longer (1-3 days) exposures. These results suggest that the increase in size of the hydrophilic part of the detergent brings about moderation in that action of the detergent which is dependent on the rate of diffusion, but the other biologically important properties of the detergent--solubilization and inactivation abilities--were not changed considerably by the increase. It is probably the size of the hydrophobic part

which is important in enzyme-inactivating properties of detergent.

The antagonist, alprenolol, was attached in a single reaction step by a sulfidic bond to sulfhydryl-sepharose. The resulting affinity resin has very low non-specific adsorption and is highly effective in the purification of  $\beta$ -adrenergic receptors from solubilized membranes.

Resting human lymphocytes were stimulated to initiate DNA synthesis by divalent mercury ions or by the divalent organomercurial 1, 4-bismercury-3, 4-dihydroxybutane. Monovalent methylmercury was ineffective, as was mercury-substituted dextran, a polyvalent compound in which mercury atoms are farther apart than in divalent butane derivative. Apparently, to stimulate DNA synthesis, the surface proteins must not only be crosslinked but also brought into close proximity by the stimulating agent.

Cu(II) rapidly oxidizes hemoglobin as a result of binding to hemoglobin at a specific site some distance from the heme. Studies of the early time course of this reaction by visible and electron spin spectroscopy indicate that there is a conformational change, which perturbs the Cu(II) binding site, that is a necessary prerequisite for the oxidation of hemoglobin. Studies with hemoglobin not in the process of being oxidized indicate that the conformation of hemoglobin which is oxidized is in equilibrium with another conformation. The properties of these two conformations are being studied to delineate the structural differences between both of these conformations and what role they may play in the functional oxygenation of hemoglobin.

The rate of hemolysis of human erythrocytes has been found to decrease as a function of subject age. In order to explain the origin of this decrease, we have found it necessary to determine what factors control the rate of hemolysis. We have been able to alter the rate of hemolysis in vitro by changing the cholesterol content of the membrane, cross linking amino groups by glutaraldehyde and altering the protein network involving spectrin which covers the inside surface of the membrane. All three of these factors alter the elastic properties of the membrane and imply that the rate of hemolysis may be determined by the mechanical resistance of the membrane to the osmotic pressure. In order to determine whether the decrease in the rate of hemolysis as a function of age is also due to a change in the properties of the membrane, we have studied the lipid composition of the erythrocyte membrane as a function of age. We find that the cholesterol content per cell and the cholesterol per phospholipid ratio both increase in older individuals. This ratio also correlates with the rate of hemolysis. It therefore seems that at least part of the change in the rate of hemolysis as a function of age can be explained by a change in the cholesterol content of the erythrocyte membrane.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00044-05 LCMB																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less)  Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">G. L. Eichhorn</td> <td style="width: 35%;">Chief, LCMB</td> <td style="width: 15%;">LCMB NIA</td> </tr> <tr> <td>OTHER:</td> <td>J. J. Butzow</td> <td>Commissioned Officer</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>P. Clark</td> <td>Research Chemist</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>Y. A. Shin</td> <td>Research Chemist</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>E. Tarien</td> <td>Chemist</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>S. Zimmerman</td> <td>Research Chemist</td> <td>LCMB NIAMDD</td> </tr> </table>			PI:	G. L. Eichhorn	Chief, LCMB	LCMB NIA	OTHER:	J. J. Butzow	Commissioned Officer	LCMB NIA		P. Clark	Research Chemist	LCMB NIA		Y. A. Shin	Research Chemist	LCMB NIA		E. Tarien	Chemist	LCMB NIA		S. Zimmerman	Research Chemist	LCMB NIAMDD
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COOPERATING UNITS (if any) Department of Chemistry, Purdue University; Department of Biology, Johns Hopkins University; Department of Physiology, University of Toronto; Laboratory of Molecular Biology, NIAMDD																										
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology																										
SECTION Section on Inorganic Biochemistry																										
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TOTAL MANYEARS: 6.5	PROFESSIONAL: 4.0	OTHER: 2.5																								
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SUMMARY OF WORK (200 words or less - underline keywords)  This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions <u>metal ions</u> are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) the effects of metal ions on the structure of <u>nucleic acids</u> , <u>nucleoproteins</u> and chromatin; (2) age-related changes in chromatin structure; (3) aging of <u>ribosomes</u> ; (4) the mechanism of involvement of <u>aluminum</u> in <u>Alzheimer's disease</u> ; (5) <u>crosslinking</u> of nucleic acid strands by metal ions; (6) the effects of metal ions on RNA polymerase; (7) metal ions and cellular aging.																										

GRC/LCMB-175



## Project Description:

Z01 AG 00044-05 LCMB

Objectives: (1) To study effects of metal ions on the structures involved in the replication, transcription, and translation of genetic information transfer, (2) to determine how errors can be introduced into genetic information transfer by metal ions, and how these errors may affect aging, (3) to understand the interactions of proteins and nucleic acids and their mediation by metal ions, (4) to understand the role of metal ions in toxicity and aging, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to elucidate structural and functional changes in genetic information transfer that accompany aging.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by optical rotatory dispersion, circular dichroism, and uv spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance, and electron spin resonance techniques to determine interaction sites. (2) Chromatin structure is analyzed by means of model systems to determine which structural features are responsible for its function. (3) Physical chemical techniques are employed to study age changes in chromatin. (4) Effects of metal ions on nucleic acids and nucleoprotein are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in information content. (5) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (6) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated. (7) Age changes in ribosomal fidelity are investigated by stressing the ribosomes with high concentrations of metal ions and with antibiotics.

## Major Findings:

A. Age-related changes in chromatin structure. Recent studies on the structure of chromatin indicate that it consists of DNA bound tightly to a combination of H2A, H2B, H3 and H4 histones in so-called nucleosomes, which are bridged by DNA bound by histone H1. The nucleosomes can be pictured as strings on a bead. The chromatin structure can be readily probed by enzymes that hydrolyze DNA, e.g., micrococcal nuclease, which indicate that different portions of the chromatin DNA have different accessibility. Thus the bridging DNA is most readily attacked, leaving the nucleosomes, which can then be further broken down into core and linker regions.

We have previously found very significant differences in the early reaction rates of young (8-9 months) and old (22-27 months) chromatin with micrococcal nuclease. Equilibrium experiments, in which the chromatin is treated with various concentrations of enzyme, lead to extents of degradation that are quite similar for young and old chromatin, within the limits of variability in chromatin of the same age. From these results we cannot conclude that any age differences exist in the number of nucleosomes or in the length of the spaces between the nucleosomes. The difference in the early reaction rates, which we have confirmed, thus indicates that the only age change is in the structure of the bridging DNA complex.



Recently Dr. E. Moudrianakis of Johns Hopkins University has isolated a protease from rat liver chromatin that cleaves histone H2A in a very specific way so that the 15 amino acids on the C-terminal of the protein are removed. In collaborative work with Dr. Moudrianakis it has been found that the activity of this enzyme is greater in old than in young rat preparations. The biological significance of this enzyme is still unknown, but, from the fact that it serves to modify a histone, one can guess that it may be involved in genetic regulation. However, the results and conclusions with this enzyme are preliminary and subject to further study.

Since earlier studies have indicated that age changes in chromatin are associated with metal ion interactions, we are investigating the effect of metal ions on the structure of chromatin by the use of circular dichroism. Very high concentrations of NaCl produce drastic changes in the CD spectrum, but  $\text{CaCl}_2$  produces similar changes at much lower concentrations which are not explained by differences in ionic strength, and which therefore indicate that  $\text{Ca}^{2+}$  binds to specific sites in the chromatin.

B. Reaction of aluminum with DNA. An observation of potentially great interest in aging research has been the discovery by Dr. D. Crapper of the University of Toronto that the brains of those who have died of Alzheimer's disease contain very high local accumulations of aluminum. Aluminum also induced lesions similar to those of Alzheimer's disease in animal brains. Though there is some dispute about these findings in that Wisniewski believes that aluminum accumulates in brain generally with age, it is obvious that this accumulation, whether or not it is related to Alzheimer's disease, could be of importance. The discovery in Dr. Crapper's laboratory that aluminum is associated with chromatin in the cell therefore suggests that the site responsible for the effect of aluminum may be DNA. In our previous studies of metal-DNA interaction we have neglected aluminum and are now making up the deficit through collaboration on this project with Dr. D. Crapper and S. Karlik of the University of Toronto.

Results to date indicate that aluminum does bind to DNA and has dramatic effects on its structure. Studies on the heat denaturation of DNA in the presence of Al show that low concentrations of Al increase the melting temperature ( $T_m$ ), high concentrations decrease it considerably, and intermediate concentrations produce a biphasic melting curve that contains  $T_m$ 's both higher and lower than those of DNA. Low Al concentration melting is partially temperature reversible and high Al concentration melting is highly reversible through addition of EDTA or high salt. These results indicate that Al forms two types of complexes with DNA, of two different stabilities and structures, but both of these form crosslinks between the DNA strands. It is tempting to suggest that such crosslinks could be responsible for the Alzheimer lesions, but of course much needs to be done to verify such a possibility.

The relative proportion of the two Al complexes can be determined by measuring the temperature reversibility of denaturation of poly d(A-T) in the presence of Al. Since poly d(AT) is readily renatured in the absence of Al, the decrease in renaturability is a measure of the concentration of the more stable complex.

Circular dichroism measurements indicate that Al causes a conformational change from the B to the C structure.

C. The effect of metal ions on RNA polymerase. The genetic message of the DNA of chromatin is transcribed into RNA through catalysis of the enzyme RNA polymerase, which requires activation by  $Mg^{2+}$ ,  $Mn^{2+}$ , or  $Co^{2+}$ . We have previously shown that in the presence of  $Mg^{2+}$  or  $Co^{2+}$  only ribonucleotides are incorporated into RNA, but with  $Mn^{2+}$  deoxynucleotides are also incorporated, thus creating errors. We have been pursuing the hypothesis that the differences in the effects of these metals lie in their influence on the conformation of the RNA polymerase. We had observed that the manganese complex of E. coli RNA polymerase melts at a different temperature from that of the cobalt and magnesium enzymes.

We have studied the effect of ribonucleoside and deoxynucleoside triphosphate on the melting curves of the three metal complexes of the enzyme. There is no detectable difference between the deox- and ribo-nucleosides in their effect on the enzyme denaturation in the presence of the metals. Therefore, it seems that the error produced by  $Mn^{2+}$  may not be due to the influence of the substrates on enzyme conformation.

The effect of the metals themselves on the enzyme conformation depends primarily on the concentration of the metal ions; the higher the metal ion concentration, the lower the denaturation temperature. There is a variability in the relative magnitude of the effects of  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Co^{2+}$  at different concentrations, but in the concentration range for the polymerase activation the dichotomy between  $Mn^{2+}$  and  $Co^{2+}$ ,  $Mg^{2+}$  persists.

The spectra of RNA polymerase and its metal complexes are very similar, but careful analysis does detect some small differences, with the greatest difference between the metal-free enzyme and the  $Mn^{2+}$  enzyme, with the  $Co^{2+}$  and  $Mg^{2+}$  enzymes intermediate.

D. Age changes in fidelity of protein synthesis. Errors in protein synthesis can result from the presence of too high concentrations of metal ions or from the introduction of antibiotics into the protein synthesizing system. We have previously shown that the concentrations of metals that produce errors will also cause mispairing of bases, so that the errors may be produced by mistaken identity in codon-anticodon interaction. Errors induced by antibiotics may reflect conformational changes induced in the ribosomes.

Higher concentrations of metal ions are required to produce errors in protein synthesis with mammalian ribosomes than with bacterial ribosomes. It appears then that mammalian ribosomes are better made to withstand stress than bacterial ribosomes. From this observation it becomes reasonable to use stress from increasing concentrations of metal ions to test the ability of ribosomes to maintain fidelity of translation. In this way old and young ribosomes can be compared. Stress on the ribosomes by the addition of antibiotics can also be used to detect differences in ribosomal fidelity due to aging.

With a polyuridylic acid message phenylalanine is correctly incorporated into protein, and mistranslation of one letter on the codon causes leucine incorporation. We therefore carried out double labeled experiments using [ $^{14}\text{C}$ ]Phe-tRNA and [ $^3\text{H}$ ]Leu-tRNA charged with their respective amino acids and determined incorporation rates of these amino acids into polymerized molecules. We prepared ribosomes from the livers of 6-month and 12-month old male rats as well as cytosol protein fractions to be used as a source of enzymes for tRNA acylation and elongation factors. Since we expected that differences in fidelity could be small, rates of incorporation were carefully measured during the linear phase of the reaction. The measurement of single incorporation points was deemed inaccurate.

Misincorporation of leucine with rat liver ribosomes was in fact observed, and linear reaction rates could be followed for both Phe and Leu incorporation. Our results so far show that old ribosomes exhibit diminished incorporation rates for both Phe and Leu, but that the important ratio of Leu:Phe remains rather similar, of the order of 0.05, for the small number of animals tested so far.

Challenging the rat liver ribosome-poly(U) system with the antibiotic, paromomycin, led to a ratio Leu:Phe in the range of 0.15. Again, even at this high level of misincorporation, we were unable to obtain consistent age differences. These results could indicate that fidelity of protein synthesis is not altered by aging either through codon-anticodon mispairing or through conformational changes in the ribosome. However, it may be that the changes are too subtle for us to have detected, and we intend to fine-tune the system further.

E. The effect of metal ions on polynucleotide structure. A long-standing concern of this laboratory has been the interactions of metal ions with nucleic acids. These interactions are important in part because of the participation of metal ions in the physiological processes that involve the nucleic acids, and in part because metal ion reactions can be used to probe the structure and function of the biological molecules.

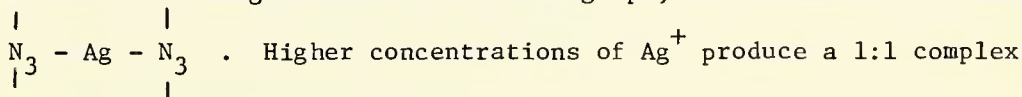
Many of the reactions of metal ions with polynucleotides serve to disorganize ordered structures, but in other cases the metal ions can produce order from disorder. Such is apparently the case with  $\text{Ag}^+$  ions, which produce a high degree of organization by reacting with unordered forms of polyuridylic acid [poly(U)] and polyinosinic acid [poly(I)].

Poly(U) characteristically has a low tendency to produce an ordered configuration and requires high salt and low temperature for association to a double helix. Yet low concentrations of  $\text{Ag}^+$  ions produce highly organized poly(U) structures that are stable beyond  $100^\circ$ . The tendency of  $\text{Ag}^+$  ions to form linear bonds with coordination number two and to bind exclusively to bases is probably responsible for their ability to organize poly(U). Complex formation is highly cooperative; i.e., the binding of one  $\text{Ag}^+$  promotes further binding, and the stability constants are high. The structures of the complexes were elucidated by ultraviolet spectrophotometry, circular dichroism, pH dependence and proton release studies, as well as X-ray fiber analysis. Stoichiometry

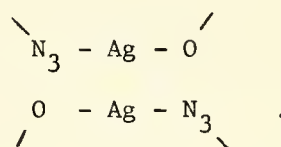


was determined by the method of continuous variation.

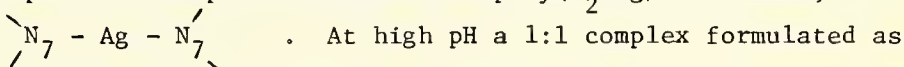
Poly(U) forms two double helical complexes with Ag. A 2:1 complex, poly (U<sub>2</sub>-Ag), is formed at low Ag concentration and high pH, and contains the structure



formulated as poly (U<sub>2</sub>Ag<sub>2</sub>), with the structure



Poly(I) also requires drastic conditions to promote ordered structures, but Ag<sup>+</sup> complexes of poly(I) were investigated in the same manner as those of poly(U). At low pH a 2:1 complex formulated as poly(I<sub>2</sub>-Ag) is formed, with the structure



poly(I<sub>4</sub>Ag<sub>4</sub>) is produced with a structure in which each Ag<sup>+</sup> is bound to N<sub>1</sub> of one base and O<sub>6</sub> of another base to form a ring (-N-Ag-O-)<sub>4</sub>. This structure is similar to the 4-stranded poly(I) structure produced by very high salt concentration, except that Ag bonds displace hydrogen bonds.

#### Significance to Biomedical Research and to the Program of the Institute:

The studies on the age changes in chromatin are of obvious relevance. The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. The possible relationship between aluminum accumulation and Alzheimer's disease and the discovery that the aluminum is bound to chromatin have emphasized the importance of studies on metal interaction with nucleic acids and chromatin. An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc. is essential to an understanding of cellular aging. We are particularly interested in studies that show how information transfer can go wrong. Metal ions are presumably not responsible for the primary events that cause aging but we believe that they may be important factors in determining individual and geographic differences in the aging process.

Proposed Course: Work will continue to determine the structure of the complexes of DNA with Al. Since aluminum is subject to complex equilibria involving hydroxide ions, we shall try to determine which Al species may be responsible for the interaction. Since the effects of Al on the DNA of chromatin are likely to have consequences on RNA synthesis, we shall also determine the effect of Al on RNA polymerase. We have already begun to test whether Al degrades RNA, as a variety of other metals do.



The age differences in rat liver chromatin have been obtained on chromatin prepared in such a way that it is not completely soluble but retains its native structure more than soluble chromatin. We have previously observed that soluble chromatin undergoes no "age" changes in WI-38 cells. We want now to determine whether or not soluble rat liver chromatin is subject to the same age changes as the relatively insoluble chromatin.

In an effort to determine how metal ions are involved in chromatin structure physical chemical studies on the binding of a variety of metal ions to chromatin will be carried out.

Experiments with Dr. E. Moudrianakis will continue to determine what the significance of the histone H2A hydrolyzing enzyme may be to age changes in chromatin.

Studies will continue into stressing ribosomes with the object of determining whether fidelity in protein synthesis changes with age.

We shall carry out physical chemical studies with RNA polymerase to pinpoint the differences in the structure of the enzyme induced by different metal ions which are responsible for the ability, or the lack of ability, to distinguish between ribo- and deoxy-nucleotides.

We shall continue our studies on the effects of metal ions on nucleic acid and nucleoprotein structure.

#### Publications:

Eichhorn, G.L., Rifkind, J.M., and Shin, Y.A.: The effect of copper on nucleic acid and nucleoprotein conformation. Adv. Chem. 162: 304-311, 1977.

Eichhorn, G.L.: Bioinorganic Chemistry. In Lapedes, D.N. (Ed.): McGraw-Hill Encyclopedia of Science and Technology. New York, McGraw-Hill, 1977, pp. 216A-216B.

Eichhorn, G.L.: Aging, genetics and the environment: Potential of errors introduced into genetic information transfer by metal ions. Mech. Ageing Dev., in press.

Srivastava, R.C., Froehlich, J., and Eichhorn, G.L.: The effect of platinum binding on the structure of DNA and its function in RNA synthesis. Biochimie, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00046-08 LCMB														
PERIOD COVERED October 1, 1977 to September 30, 1978																
TITLE OF PROJECT (80 characters or less)  Polymers as Biological Reagents																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. Pitha</td> <td style="width: 35%;">Research Chemist</td> <td style="width: 15%;">LCMB NIA</td> </tr> <tr> <td rowspan="3">Other:</td> <td>M. Akashi</td> <td>Visiting Fellow</td> <td>LCMB NIA (EOD 04/24/78)</td> </tr> <tr> <td>L. Blob</td> <td>NIH Postdoctorate Fellow</td> <td>LCMB NIA (DOD 01/29/78)</td> </tr> <tr> <td>K. Kociolek</td> <td>Visiting Fellow</td> <td>LCMB NIA (DOD 12/02/77)</td> </tr> </table>			PI:	J. Pitha	Research Chemist	LCMB NIA	Other:	M. Akashi	Visiting Fellow	LCMB NIA (EOD 04/24/78)	L. Blob	NIH Postdoctorate Fellow	LCMB NIA (DOD 01/29/78)	K. Kociolek	Visiting Fellow	LCMB NIA (DOD 12/02/77)
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	K. Kociolek	Visiting Fellow	LCMB NIA (DOD 12/02/77)													
COOPERATING UNITS (if any)  None																
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology																
SECTION Section on Macromolecules																
INSTITUTE AND LOCATION NIH, NIA, Baltimore, Maryland 21224																
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0														
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SUMMARY OF WORK (200 words or less - underline keywords)  <p>             In this project chemically reactive and water soluble <u>macromolecules</u> have been synthesized and their effects on mammalian cells in culture studied, the main emphasis being on the study of their interaction with <u>cell membrane</u>. <u>Polysaccharides</u>, dextran and inulin, which are neither bound nor consumed by cells, were used as carriers for various chemically reactive groups. The resulting macromolecules have been tested <u>in vitro</u> on mouse erythroleukemic cells, on human fibroblast and on human lymphocytes. In addition to previously prepared and studied compounds the following new reactive groups were used: a) <u>Sulfhydryl</u> groups, which are reducing agents for the surface of cells and furthermore bind to exposed cysteine residues and thus lead to a coating of cells with polysaccharides. Furthermore, sulfhydryl substituted macromolecules were used for chemical attachment of the drugs which contained allyl groups to polysaccharide carriers. b) <u>Residues of detergent Triton X-100</u> - the resulting polysaccharide derivatives were used as mild acting surfactants in the permeabilization of cells <u>in vitro</u>.           </p>																

## Project Description:

Objectives: Macromolecules have only limited potential to penetrate into the inside of cells; thus they represent suitable reagents for the cell surfaces. The cellular surface is an ideal location for drug action; if a suitable macromolecular and cell selective drug can be obtained, a pharmacological intervention can be made without burdening the interior of any cell organism with foreign compounds. The ultimate intent of the project is to create a rational basis for the design of such drugs. In these early stages the objective of research is to obtain biologically active macromolecules and correlate their activity with their structure.

Methods Employed: The study requires both chemical and biological methods. Methods for attachment of small molecular weight compounds to polysaccharides were investigated and the stability of the attachment measured under physiological conditions. These compounds were then tested on (a) mouse erythroleukemic cells which grow in suspension and thus are easily obtainable in bulk, (b) human fibroblast cells, which grow in monolayers and are thus obtainable only in smaller amounts and (c) human lymphocytes.

Major Findings: Stimulation of lymphocytes. Resting human lymphocytes were stimulated to initiate DNA synthesis by divalent mercury ions or by the divalent organomercurial 1, 4-bismercury-3, 4-dihydroxybutane. Monovalent methylmercury was ineffective as was mercury-substituted dextran, a polyvalent compound in which mercury atoms are farther apart than in divalent butane derivative. Apparently, to stimulate DNA synthesis, the surface proteins must not only be crosslinked but also brought into close proximity by the stimulating agent.

Effects of transformation and aging on the cell surface of human fibroblasts. Nucleic acids in the presence of carcinogens are strongly adsorbed to the surface of cells in vitro. Using polyuridyate and proflavine, it was shown that the adsorption occurs on the anionic polysaccharides on the cell surfaces and that the amount of these surface components may be measured in that way. Investigation of a large variety of normal and genetically defective human cells from young and old donors and the same cells transformed by SV40 indicated that all these cells have the same amount of surface anionic polysaccharides when compared at the same cell density. Human fibroblast aged in vitro had, compared to all the other cells, higher amounts of surface receptors.

Effects of macromolecular detergent on membranes and cells. Residues of Triton X-100 were attached to the polysaccharide inulin. The resulting detergent was effective in the solubilization of proteins and phospholipids from membranes of human erythrocytes and led to a release of reverse transcriptase activity from the membrane enveloped virions of murine leukemia. The Triton X-100 inulin derivative inactivated  $\beta$ -adrenergic receptors from frog erythrocytes in a dose related manner similar to the inactivation produced by Triton X-100. On the other hand, digitonin, a detergent containing a bulkier hydrophobic group, did not cause inactivation. On the basis of its Triton X-100 content the inulin derivative was found to be less detrimental to the growth of murine erythroleukemic cells in vitro than Triton X-100 alone when short (2-4 hr) exposure was used, but this difference disappeared at longer (1-3 day)

exposures. These results suggest that the increase in size of the hydrophilic part of the detergent brings about moderation in that action of the detergent which is dependent on the rate of diffusion but the other biologically important properties of the detergent--solubilization and inactivation abilities--were not changed considerably by the increase. It is probably the size of the hydrophobic part which is important in enzyme-inactivating properties of the detergent.

Affinity resin for the purification of  $\beta$ -adrenergic receptors. The antagonist, alprenolol, was attached in a single reaction step by a sulfidic bond to sulfhydryl-sepharose. The resulting affinity resin has very low non-specific adsorption and is highly effective in the purification of  $\beta$ -adrenergic receptors from solubilized membranes.

Significance to Biomedical Research and Program of the Institute. Macromolecules differ profoundly from low molecular weight compounds in their biological potentials. The present studies aim to use these differences in the investigation of the cell surface, a cellular component which is supposed to reflect closely the state and age of the cell itself.

Proposed Course of Project. By directed synthesis and study of the basic biological effects of macromolecules, it is hoped to gain knowledge necessary for the design of practically useful compounds; e.g., antiviral drugs and drugs that inhibit virus specific enzymes, reagents that react with the cell surface and specific groups on the cell surface, and reagents that can detect surface charge.

#### Publications:

Pitha, P.M. and Pitha, J.: Polynucleotide analogs as inhibitors of DNA and RNA polymerases. Pharmacol. Ther. [A] 2: 247-260, 1978.

Pitha, J.: Reagents specific for cell surface components. Eur. J. Biochem. 82: 285-292, 1978.

Pitha, J., Wilson, S.H. and Pitha, P.M.: A vinyl polymer with purine residues deficient in base pairing inhibits murine leukemia virus replication. Biochem. Biophys. Res. Commun. 81: 217-223, 1978.

Vengris, V.E., Pitha, P.M., Sensenbrenner, L.L. and Pitha, J.: Polymeric drugs: Direct compared with indirect inhibition of leukemia virus replication in mice. Mol. Pharmacol. 14: 271-277, 1977.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00047-08 LCMB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Relation of Structure and Function in Hemoglobin		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: J. M. Rifkind OTHER: K. Araki H. Yeh S. Chiang	Research Chemist Visiting Fellow Research Chemist Visiting Fellow	LCMB NIA LCMB NIA LC NIAMDD LC NIAMDD
COOPERATING UNITS (if any) Duke University Marine Laboratory; Johns Hopkins University; University of Minnesota; Laboratory of Chemistry, NIAMDD		
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology		
SECTION Section on Inorganic Biochemistry		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5
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<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to <u>hemoglobin</u> and the transport of oxygen to the tissues. The project also focuses on ways in which these functions are impaired and change with age. We have also studied the mechanisms involved in the <u>oxidation</u> of hemoglobin. Oxidation effects <u>oxygen transport</u> because it produces non-functional hemoglobin, which no longer binds oxygen. We have extended these studies to include an investigation of the stability of the <u>erythrocyte</u> as measured by <u>osmotic fragility</u> and <u>rates of hemolysis</u> . Changes in these parameters have been observed as a function of age and studies have been initiated to explain the origin of these age-related changes.		

GRC/LCMB-185

## Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues. (4) To elucidate age-related changes in the composition and functional properties of the erythrocyte.

Methods Employed: Various preparative procedures are used to purify hemoglobin and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis, are used to analyze for various erythrocyte components. The oxygenation and oxidation of hemoglobin are investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances are studied by equilibrium dialysis. Electron spin resonance is used to observe paramagnetic Cu(II) bound to protein, and to follow the changes in paramagnetic species found as hemoglobin is oxidized. The extent of hemolysis and the rate of hemolysis of various samples of red cells are observed under various conditions with and without the addition of various substances.

## Major Findings:

A. Oxidation of hemoglobin by copper. We have previously found that Cu(II) oxidizes hemoglobin as a result of binding to hemoglobin at a specific site on the protein moiety some distance from the heme. In order to explain how the electron can be transferred between the Cu(II) and Fe(II) atoms, we have been studying the early time course of this reaction by both visible and electron spin spectroscopy. The visible absorption studies indicate that there is a 40 msec lag in the oxidation. The ESR studies indicate that Cu(II) is completely bound to hemoglobin in < 10 msec. However, this very rapid binding is followed by an alteration of the Cu(II) ESR spectrum which is indicative of a conformational change which perturbs the Cu(II) binding site. Our studies indicate that this conformational change is a necessary prerequisite for the oxidation of hemoglobin and, therefore, helps to explain how the electron is transferred between Cu(II) and Fe(II).

B. An equilibrium between two conformations detected by the Cu(II) ESR spectrum. Studies with hemoglobin not in the process of being oxidized indicate that the conformation of hemoglobin which is rapidly oxidized is in equilibrium with another conformation. The equilibrium between these two forms of hemoglobin depends on the liganded state of hemoglobin, the pH, and the addition of metal ions. These conformations do not seem to be identical to the extensively studied liganded and unliganded conformations of hemoglobin. We are in the process of studying the properties of these two conformations and find that the reactive sulfhydryl group located near the heme is much more reactive in one of these two conformations. The conformation equilibrium plays a central role in the oxidation of hemoglobin and may also be involved in the functional oxygenation of hemoglobin.

C. The rate of hemolysis. We have previously found that in older individuals the erythrocytes are more fragile but that the rate of hemolysis is slower. In vitro incubation studies demonstrate that the rate of hemolysis and the fragility curve measure two different properties of the cells. The more frequently studied fragility curve reflects the biconcave shape of the erythrocyte and the swelling of the cell in hypotonic salt solution. On the other hand, the rate of hemolysis may more directly reflect the membrane properties of the cell. We have now varied various components of the erythrocyte membrane in order to understand what factors determine the rate of hemolysis. No effect on the time course of hemolysis was observed when the membrane sulfhydryl groups were reacted with N-ethylmaleimide or the negative charges were removed by pronase. Blocking the water channel by para-chloromercuribenzenesulfonate dramatically prolongs the lag due to swelling observed in the hemolysis reaction but has a relatively small effect on the actual rate of hemolysis. Effects on the rate of hemolysis are observed by altering the cholesterol content of the membrane, crosslinking amino groups by glutaraldehyde and altering the protein network involving spectrin which covers the inside surface of the membrane. All three of these factors alter the elastic properties of the membrane and imply that the rate of hemolysis is affected by the mechanical resistance of the membrane to the osmotic pressure.

D. The effect of age on the cholesterol content of the erythrocyte membrane. The finding that erythrocytes of older individuals have a slower rate of hemolysis and that increasing the cholesterol/phospholipid ratio of the erythrocyte membrane decreases the rate of hemolysis suggested that the aging effect may be related to a change in the lipid composition of the membrane. Studies on adult males 27-95 years of age from the longitudinal program indicates that the cholesterol level per cell does increase as a function of age ( $r = 0.42$ ;  $P < 0.025$ ) and that even greater significance is found for the molar ratio of cholesterol to phospholipid ( $r = 0.47$ ;  $P < 0.005$ ). On the other hand, no significant change in the phospholipid level is observed. We also find a very significant inverse relationship between the molar ratio of cholesterol to phospholipid and the rate of hemolysis ( $r = -0.69$ ;  $P < 0.001$ ). These results indicate that at least part of the observed change in the rate of hemolysis as a function of age is due to the higher cholesterol level of the erythrocyte in older individuals. Since the erythrocyte membrane cholesterol has been shown to be at equilibrium with the plasma cholesterol, the increase in membrane cholesterol must reflect some change in the distribution of cholesterol in the plasma. However, no correlation was observed between the erythrocyte cholesterol to phospholipid ratio and the total plasma cholesterol. Plasma cholesterol consists of esterified and free cholesterol associated with proteins in different lipoprotein fractions. The erythrocyte results reflect a change in the distribution of plasma cholesterol resulting in a greater transfer of cholesterol to the erythrocyte membrane. This change in the erythrocyte cholesterol may therefore perhaps parallel a generalized increase in cholesterol and decreased elasticity of other membranes as well.

Significance to Biomedical Research and Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the



cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to maintain the integrity of the erythrocyte and to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin in circulation. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues.

Proposed Course of the Project: (1) We plan to study the oxygenation of whole blood from individuals of various ages and try to correlate any observed changes with alterations in the erythrocyte composition and/or the hemoglobin molecule. (2) We plan to investigate the possible physiological significance of the reported zinc-induced increase in the oxygen affinity of hemoglobin. (3) We plan to further investigate the binding site for metal ions on hemoglobin and the mechanism whereby the bound metal ions alter functional properties of hemoglobin. (4) We plan to study the properties and composition of the erythrocyte membrane and how they change with age, both as a measure of the stability of the erythrocyte and possible effects on the transport of oxygen in and out of the cell.

Publications:

Rifkind, J.M. and Heim, J.M.: Interaction of zinc with hemoglobin: Binding of zinc and the oxygen affinity. Biochemistry 16: 4438-4443, 1977.

Rifkind, J.M., Keyes, M.H., and Lumry, R.: Linkage between the binding sites for zinc and oxygen on sperm whale myoglobin. Biochemistry 16: 5564-5568, 1977.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00087-05 LCMB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Mechanism of the Parental Age Effects														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">E. L. Schneider</td> <td style="width: 30%;">Medical Officer, PHS</td> <td style="width: 30%;">LCMB NIA</td> </tr> <tr> <td>OTHER:</td> <td>D. Kram</td> <td>Staff Fellow</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>G. Bynum</td> <td>Medical Officer, PHS</td> <td>LCMB NIA</td> </tr> </table>			PI:	E. L. Schneider	Medical Officer, PHS	LCMB NIA	OTHER:	D. Kram	Staff Fellow	LCMB NIA		G. Bynum	Medical Officer, PHS	LCMB NIA
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COOPERATING UNITS (if any) University of California Livermore Laboratory; Department of Microbiology, Notre Dame University														
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology														
SECTION Section on Cellular Aging and Genetics														
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224														
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SUMMARY OF WORK (200 words or less - underline keywords) With increasing <u>parental age</u> , there is an exponential increase in the frequency of children born with <u>chromosomal disorders</u> . Most studies indicate that this effect is chiefly due to <u>maternal aging</u> . We have developed a <u>mouse model</u> for examining this maternal age effect and have demonstrated a highly significant increase in the frequency of chromosomally abnormal mouse fetuses with maternal aging. Studies conducted on mice have indicated that neither <u>immunologic deficiency</u> nor <u>genetic predisposition</u> appear to play a strong role in the maternal age-related increase in chromosomally abnormal offspring. Current research is directed at examining other proposed etiologic agents for the maternal age effect as well as analyzing the potential role of <u>paternal aging</u> . The latter area is being approached by measuring the genetic complement of sperm samples obtained from members of the Baltimore Longitudinal Study.														

GRC/LCMB-189

### Project Description:

Objectives: It has been clearly established that with increased maternal age there is a greatly increased risk of children being born with chromosomal disorders. Despite considerable speculation about the cause of this maternal age effect, research to delineate the mechanisms of this effect has been limited by the practical as well as ethical considerations of human experimentation. This problem has been approached in this laboratory by utilizing the mouse as an animal model since it has been demonstrated that with increased mouse maternal age there is an increased frequency of chromosomally abnormal fetuses. Further studies were directed at examining the genetic and immunological components of the maternal age effect. Although minor differences were observed in the age-related increase in chromosomally abnormal offspring between various mouse inbred strains, no substantial genetic component could be defined. Immune deficiency also did not appear to significantly affect the frequency of chromosomally abnormal fetuses. The next approach to examining the mechanisms of the maternal age effect will encompass an investigation of the effect of infectious agents.

Recent evidence indicates that advanced paternal age may also contribute to the increased frequency of chromosomally abnormal offspring with advanced parental age. This paternal age component can be directly evaluated by examining the chromosomal complement of human sperm samples derived from volunteer members of the Baltimore Longitudinal Study.

Methods Employed: (1) "Germ-free" animals are currently aging at Dr. Pollard's laboratory at Notre Dame University. The frequency of chromosomally abnormal embryos will then be compared in litters from young and old "germ-free" and control mice. (2) Sperm samples obtained from young and old volunteer members of the Baltimore Longitudinal Study were sent to Dr. Barton Gledhill of the University of California Lawrence Livermore Laboratories for flow microfluorometric analysis. With flow microfluorometric analysis, the DNA contents of individual sperm can be measured with great accuracy. (3) Sperm samples from longitudinal subjects were also placed on slides and stained with the appropriate fluorescent stains to detect fluorescence of the Y chromosome. These Y bodies are an indicator of the number of Y chromosomes present in a single sperm. The number of double Y bodies indicating the presence of two Y chromosomes reflects the overall incidence of chromosomal aneuploidy.

Major Findings: (1) Manuscripts are now in press detailing our early findings of a lack of a strong genetic or immunological component to the maternal age effect. (2) Flow microfluorometric analyses indicate that this technique can be applied to examining chromosomal aneuploidy in human sperm samples. Results obtained by flow microfluorometry appear to correlate well with staining for Y bodies.

### Significance to Biomedical Research and the Program of the Institute:

Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is considerably higher if one considers that over one-half the spontaneous abortions that occur

during pregnancy are due to chromosomal abnormalities. With increasing maternal age, the risk of having a child with a chromosomal abnormality, such as Down's syndrome (mongolism), increases dramatically. A mother at age 45 or above has a 100-fold greater chance of having a child with this syndrome than a mother aged 15 to 20. It is, therefore, of great clinical importance that insight into the mechanisms of this maternal age effect be obtained.

The mouse has proved to be an appropriate animal model for studying this maternal age effect since a marked increase in the frequency of chromosomally abnormal embryos has been observed with increasing maternal age. A survey of mouse inbred strains did not reveal significant differences in the maternal age effect between strains but instead indicated that the maternal age effect was present to a similar degree in all strains examined. These results suggest a lack of a strong genetic component of the maternal age effect. Similarly, comparison of the frequency of chromosomally abnormal embryos between mice in which immune incompetence was induced and controls did not reveal a significant increase in aneuploidy as a function of altered immunity. Therefore, if the results of these studies can be applied to man, they would suggest that neither genetic predisposition nor altered immunity play a vital role in the increased frequency of chromosomally abnormal offspring born to older mothers.

Proposed Course: Studies of the maternal age effect will be continued by examining other proposed etiologic agents. The possible role of infectious agents will be assessed by analyzing the frequency of chromosomally abnormal fetuses in germ-free mice in collaboration with Dr. Morris Pollard.

Studies of the paternal age effect will continue with flow microfluorometric and cytologic analysis of sperm samples obtained from volunteer members of the Baltimore Longitudinal Study. In addition, an attempt will be made to find a mouse model for the paternal age effects seen in humans.

#### Publications:

Kram, D. and Schneider, E.L.: An Effect of Reproductive Aging: Increased Risk for Genetically Abnormal Offspring. In Schneider, E.L. (Ed.): The Aging Reproductive System. New York, Raven Press, 1978, pp. 220-237.

Kram, D. and Schneider, E.L.: Parental Age Effects. In Schneider, E.L. (Ed.): The Genetics of Aging. New York, Plenum Press, 1978, pp. 225-260.

Fabricant, J.D. and Schneider, E.L.: Studies of the genetic and immunologic components of the maternal age effect. Dev. Biol., in press.

Fabricant, J.D., Dunn, G., and Schneider, E.L.: Maternal age-related pre- and post-implantation fetal mortality: A strain survey. Mech. Ageing Dev., in press.

Z01 AG 00087-05 LCMB

Editor: Schneider, E.L. (Ed.): The Genetics of Aging. New York,  
Plenum Publ. Corp., 1978, 424 pp.

Editor: Schneider, E.L. (Ed.): Aging. The Aging Reproductive System.  
New York, Raven Press, 1978, Vol. 4, 270 pp.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00088-06 LCMB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less)  Mechanisms of Cellular Aging																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">E. L. Schneider</td> <td style="width: 30%;">Medical Officer, PHS</td> <td style="width: 30%;">LCMB NIA</td> </tr> <tr> <td>OTHER:</td> <td>D. Kram</td> <td>Staff Fellow</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>Y. Nakanishi</td> <td>Visiting Fellow</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>G. Bynum</td> <td>Medical Officer, PHS</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>P. Thorne</td> <td>Chief, Tech. Dev. Sect.</td> <td>OC NIA</td> </tr> </table>			PI:	E. L. Schneider	Medical Officer, PHS	LCMB NIA	OTHER:	D. Kram	Staff Fellow	LCMB NIA		Y. Nakanishi	Visiting Fellow	LCMB NIA		G. Bynum	Medical Officer, PHS	LCMB NIA		P. Thorne	Chief, Tech. Dev. Sect.	OC NIA
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COOPERATING UNITS (if any) W. Alton Jones Cell Science Center; School of Medicine, Johns Hopkins University; School of Public Health and Hygiene, Johns Hopkins University																						
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SUMMARY OF WORK (200 words or less - underline keywords) Our major objectives are to examine <u>cell replication</u> and <u>repair of DNA damage</u> as a function of <u>aging in human cells in vitro</u> and in <u>rat and mouse tissues in vivo</u> . A technique has been developed to <u>examine cell replication in vivo</u> in intact animals utilizing the BrdU-differential staining technique. Results obtained with this technique indicate a decline in cell replication as a function of aging. Further research will be directed not only at defining this decline in cell replication but also at investigating the mechanisms for this functional loss.  The BrdU-differential staining techniques have also been applied to examining DNA repair by analyzing the frequencies of sister chromatid exchanges (SCE) induced by various DNA damaging agents <u>in vivo</u> in mouse and rat bone marrow cells and <u>in vitro</u> in cultured human fibroblasts. In all these systems, a significant decrease in MMC-induced SCE was observed in the older cell populations. The mechanism for the altered response to DNA damage as manifested by diminished SCE is being investigated.																						

GRC/LCMB-193

## Project Description:

Z01 AG 00088-06 LCMB

**Objectives:** A decline in the proliferative capacities of certain cell populations is an important feature of human aging. Because of the practical and ethical limitations of in vivo human experimentation, considerable effort has been expended to create appropriate in vitro tissue culture systems as well as in vivo animal models for examining age-related alterations in cell replication.

There has been considerable concern expressed over whether in vitro human diploid fibroblast aging reflects in vivo human aging. To examine this, we are establishing large numbers of skin fibroblast cultures from members of the Baltimore Longitudinal Study. Colony size distributions, which have been shown to be an excellent index of in vitro proliferative ability, will be performed on all of these cultures. Since a number of in vivo studies are also performed on these subjects, we will be able to compare in vitro measurements with in vivo measurements obtained from the same individuals. Although in vitro studies of cell replication are important, they are like all in vitro work restricted by the limitations of an artificial environment. It was, therefore, vital that an in vivo technique be developed to examine cell replicative abilities as a function of age. Previous in vivo studies were performed with radioactive labeling or by examining the end result of cellular proliferation. However, the advent of new cytogenetic techniques permits direct examination of cell replication as a function of age without the need for radioisotopes. With these techniques, cell replication can be studied in several animal systems.

Another aspect of this project will be to examine the mechanisms for the age-associated decline in cell replication. One important function that is crucial to DNA replication is the ability of replicating cells to respond to DNA damage. It was, therefore, decided to investigate the ability of replicating cells to respond to DNA damage both in vitro and in vivo as a function of age.

**Methods Employed:** (1) For measurement of cell replication in vivo, young and old animals were infused intravenously with BrdU. At increasing time intervals, these animals were sacrificed and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by characteristic banding patterns. (2) By utilizing the BrdU-differential staining technique both in vitro and in vivo, one can measure the frequency of sister chromatid exchanges (SCE). This frequency reflects the response of cells to DNA damage.

**Major Findings:** (1) Further research with the BrdU-differential staining techniques indicates that this new approach to measuring cell replication kinetics can be easily utilized in vivo as well as in vitro. Mathematical models have been developed for both in vivo and in vitro cell kinetic analyses. (2) Application of these techniques to PHA-stimulated human lymphocytes in vitro and rat and mouse bone marrow cells in vivo has revealed a significant decrease in cell replication as a function of aging. In the lymphocyte system we are able to demonstrate that both decreased PHA stimulation and diminished cell replication kinetics in the stimulated population

were responsible for the decline in cell replication observed with aging. (3) Further analyses of the effect of aging on mutagen-induced SCE frequencies indicate that this observation is not limited to a specific compound. In both human diploid cells in vitro and mouse and rat cells in vivo, aging cell populations have diminished SCE induction when treated with a number of compounds ranging from alkylating compounds such as AAF and EMS to intercalating agents such as adriamycin. Examination of the kinetics for this decline in mutagen-induced SCE indicates that induced SCE frequencies remain stable during early adulthood (6-18 months in mice and rats) and then decline with further aging (19+ months). (4) Examination of MMC, cyclophosphamide and adriamycin-induced SCE in AKR mice, which display a predisposition toward malignancy and early mortality, revealed a similar decline in SCE frequencies. This decrease in mutagen-induced SCE frequencies is also present in AKR embryonic cells in vitro. F<sub>1</sub> hybrids of AKR and C57BL/6J mice have intermediate frequencies of mutagen-induced SCEs. (5) Studies of SCE induction by a number of compounds in vivo indicate that our system is a sensitive measure of in vivo mutagen and carcinogen exposure. In addition, in our in vitro human cell systems, we have demonstrated that the fluorescent light used for room lighting is capable of SCE induction. (6) Further studies with skin fibroblast cultures derived from young and old human donors indicate that neither insulin nor EGF receptors change as a function of aging and that macromolecular synthesis is unaltered in old cells.

Significance to Biomedical Research and the Program of the Institute: The application of the BrdU-differential staining technique to cell kinetic measurements has provided a new and sensitive tool to examine cell proliferation both in vitro in cultured human cells and in vivo in intact animals. These studies have already shed light on the controversy over whether cell proliferation is diminished with in vivo aging. Our results indicate a consistent decline in cell replication both in vivo and in vitro. Measurements of SCE frequencies were shown to be a sensitive indicator of DNA damage both in vivo and in vitro. Our studies in four separate systems all indicate an altered response of old cells to induced DNA damage. Since SCE appear to be closely related to chromosomal structure, these results suggest a significant alteration in chromosomal structure with cellular aging. The finding of impaired SCE formation in AKR mice at an early age indicates that alterations in chromosomal structure related to SCE may play a role in the development of malignancy which characterizes this mouse strain and leads to its early death. This mouse strain may thus be a good model system for aging research.

Proposed Course: Further studies of cellular replication will be focused on discerning the mechanisms for the decline in cell proliferation with aging. An attempt will be made to isolate defined populations of replicating cells for these studies. In addition, cells from old animals will be placed in young environments by bone marrow transplantation to lethally irradiated host animals. This should permit discrimination between intrinsic and extrinsic components to the age-related decline in cell proliferation.

Our studies of the effect of aging on SCE induction will be continued and attempts will be made to find agents capable of restoring induced SCE levels in old cells to the levels observed in young cell populations.



Differentiated cell systems will be developed to study specific age-related disorders. Among the cell systems under consideration are osteoblasts/osteoclasts (osteoporosis), epidermal keratinocytes (skin cancer) and blood vessel endothelial cells (arteriosclerosis).

Studies of skin fibroblast cultures derived from old and young human volunteer members of the Baltimore Longitudinal Study will continue with an emphasis on collaboration with outside laboratories with expertise in a variety of areas ranging from transformability of these cells with SV<sub>40</sub> virus, cell mobility, antioxidant properties, and cyclic nucleotide metabolism.

#### Publications:

Schneider, E.L., Mitsui, Y., Au, K.S., and Shorr, S.S.: Tissue-specific differences in cultured human diploid fibroblasts. Exp. Cell Res. 108: 1-6, 1977.

Schneider, E.L., Epstein, C.J., Kaback, M., and Brandes, D.: Severe pulmonary involvement in Gaucher's disease. Report of three cases and review of the literature. Am. J. Med. 63: 475-480, 1977.

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Gal, A.E., Brady, R.O., Pentchev, P.G., Furbish, F.S., Suzuki, K., Tanaka, H., and Schneider, E.L.: A practical chromogenic procedure for the diagnosis of Krabbe's disease. Clin. Chim. Acta 77: 53-59, 1977.

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Smith, J.R., Smith, O.M.P., and Schneider, E.L.: Colony size distributions as a measure of in vivo and in vitro aging. Proc. Natl. Acad. Sci. (USA) 75: 1353-1356, 1978.

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Schneider, E.L.: A new approach to measuring human biologic age: Studies on human skin fibroblasts in tissue culture. Institut de la Vie Symposium on Aging, in press.

Kram, D., Schneider, E.L., Tice, R.R., and Gianas, P.: Aging and sister chromatid exchange I. The effect of aging on mitomycin-C induced sister chromatid exchange frequencies in mouse and rat bone marrow cells in vivo. Exp. Cell Res., in press.

Schneider, E.L. and Monticone, R.E.: Aging and sister chromatid exchange II. The effect of the in vitro passage level of human fetal lung fibroblasts on baseline and mutagen-induced sister chromatid exchange frequencies. Exp. Cell Res., in press.

Schneider, E.L., Sternberg, H., Tice, R.R., Senula, G.C., Kram, D., Smith, J.R., and Bynum, G.: Cellular replication and aging. Mech. Ageing Dev., in press.

Schneider, E.L., Kram, D., Nakanishi, Y., Monticone, R.E., Tice, R.R., Gilman, B., and Nieder, M.: The effect of aging on sister chromatid exchange. Mech. Ageing Dev., in press.

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Monticone, R.E. and Schneider, E.L.: Induction of sister chromatid exchanges in human cells by fluorescent light. Mutat. Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00102-02 LCMB																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less) Comparative Mammalian Biochemistry I. Stability of Gene Control Mechanisms																						
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<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">R. Cutler</td> <td style="width: 30%;">Research Chemist</td> <td style="width: 30%;">LCMB NIA</td> </tr> <tr> <td>Other:</td> <td>R. Dean</td> <td>Staff Fellow</td> <td>LCMB NIA</td> </tr> <tr> <td></td> <td>K. Kator</td> <td>Visiting Fellow</td> <td>LCMB NIA (EOD 12/01/77)</td> </tr> <tr> <td></td> <td>T. Ono</td> <td>Visiting Fellow</td> <td>LCMB NIA (DOD 05/12/78)</td> </tr> <tr> <td></td> <td>S. Chattopadhyay</td> <td>Senior Staff Fellow</td> <td>PO NCI</td> </tr> </table>			PI:	R. Cutler	Research Chemist	LCMB NIA	Other:	R. Dean	Staff Fellow	LCMB NIA		K. Kator	Visiting Fellow	LCMB NIA (EOD 12/01/77)		T. Ono	Visiting Fellow	LCMB NIA (DOD 05/12/78)		S. Chattopadhyay	Senior Staff Fellow	PO NCI
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	S. Chattopadhyay	Senior Staff Fellow	PO NCI																			
COOPERATING UNITS (if any) Pediatric Oncology, DCT, NCI; Department of Medicine, Yale University School of Medicine; Mayo Clinic Foundation; Department of Cell Biology, Baylor College of Medicine; Cancer Research Unit, McEachern Laboratory, Univ. of Alberta.																						
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology																						
SECTION Section on Macromolecules																						
INSTITUTE AND LOCATION NIH, NIA, Baltimore, Maryland 21224																						
TOTAL MANYEARS: 3.1	PROFESSIONAL: 2.5	OTHER: 0.6																				
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SUMMARY OF WORK (200 words or less - underline keywords)  This project represents the first part of a program designed to search for the presence of <u>primary aging processes</u> and the <u>natural means</u> by which longevity evolved during the recent <u>evolutionary history</u> of man. A brief review of the general objectives and rationale of this program is presented. Present work is focused on possible <u>age-dependent relaxation of specific</u> <u>genes</u> in tissues where their expression is not expected. Evidence has been found for an age-dependent expression of $\alpha$ and $\beta$ <u>globin genes</u> in <u>liver</u> and <u>brain</u> tissues in the long-lived C57BL/6J <u>mouse strain</u> ; whereas in the short- lived AKR mouse strain, having a high incidence frequency of <u>leukemia</u> , an early expression is found only in the <u>thymus</u> , and this is limited to the C- type virus genes. More recent work has now extended this investigation to include <u>casein</u> , <u><math>\alpha</math>-fetal protein</u> and <u>mouse mammary tumor virus genes</u> .																						

GRC/LCMB-198

## Project Description:

Objectives: Essentially all known structures and functions of the mammalian organism become altered with increasing chronological age. Many of these alterations are believed to be related to the well known age-dependent decline in physiological performance and the increased onset frequency of many dysfunctions and diseases which account for much of the decline in the mental and physiological health of man. Most investigators in the medical and gerontological sciences have undertaken a compartmentalized approach in studying these time-dependent structural and functional changes, therefore limiting their area of investigation and, hopefully, the complexity of the problem.

It is now becoming clear, however, that any substantial extension in the period that general mental and physical health can be maintained requires a decrease in the rate of expression of most of the aging processes. In view of the complexity of these processes, the probability of accomplishing this objective appears to be extremely low unless primary aging processes exist which underlie most age-dependent alterations. The general long-term objective of our research program is to determine if such primary aging processes exist and, if so, how their rate of expression is controlled. The general method of approach taken is an evolutionary-comparative biochemistry of mammalian species closely related to one another--but having substantial differences in their innate capacity to maintain (as a function of chronological age) optimum mental and physical health.

Our evolutionary studies of human longevity indicating a surprisingly high rate of increased maximum lifespan potential over a relatively short time period support the concept of primary aging processes and suggest that only a few alterations of regulatory genes may have resulted in modern man's exceptionally long lifespan potential. A remarkable similarity also has been found in the physiological, biochemical and qualitative nature of age-dependent dysfunctions and diseases of mammals in general, in spite of substantial differences in their maximum lifespan potentials. These and other findings have suggested a new concept: that special anti-aging processes exist that are common to most mammalian species and act according to their levels of activity to govern the rate of expression of a common set of primary aging processes. Through a comparative biochemical approach we hope to identify these hypothetical primary aging processes and their related anti-aging processes which control their rate of expression. In effect, we are searching for the unique biological properties of man which have resulted in his exceptional ability to maintain mental and physical health.

Our current work continues to investigate the evolutionary aspects of human longevity by reviewing literature on the comparative aspects of physiology, biochemistry and molecular biology of primates and their age-dependent dysfunctions and diseases. Laboratory work centers on investigating if informational storage and transfer systems of the cell may be areas where primary aging and their related repair and protective processes may operate. This work is divided into three research projects, entitled: (I) Stability of Gene Control Mechanisms, (II) Physico-chemical Characterization of Chromatin, and (III) Species-dependent Levels of Genetic Repair and Protective Processes.



The remainder of this report concerns Project I.

The proper function of the adult mammalian organism is dependent upon maintaining the correct differentiated state of many types of cells. Any deviation from this optimum state is likely to decrease the operational effectiveness of the cell and, if of sufficient magnitude, lead to serious loss of efficiency--resulting in general dysfunctions and disease processes at the organismic level. Such dedifferentiation processes of relaxed gene expression might also be involved in the appearance of neurofibrillary tangles, senile plaques, autoimmune disease, and some types of slow virus diseases and cancers. Our present aim is to examine the age-dependent stability of the differentiated cell by determining if improper specific and general gene expression occurs in specific cell populations as a function of age.

Methods Employed: The general method used to investigate improper specific gene expression is to search for the presence of the RNA transcribed by this gene using a radioactive complementary DNA (cDNA) probe. The cDNA probe is obtained by isolation of pure messenger RNA known to code for a specific gene product and then to use this RNA as a template with reverse transcriptase enzyme to synthesize the radioactive cDNA probe. RNA is then extracted from the nuclei and/or cytoplasmic fractions of certain tissues and the presence of RNA complementary to the cDNA probes determined by DNA-RNA hybridization techniques. We are presently investigating  $\alpha$  and  $\beta$  globin, casein,  $\alpha$ -fetal protein, mouse leukemia (MuLV) and mouse mammary tumor virus (MMTV) genes. Both of the viral genes are c-type viruses, whose genomes are integrated into the normal cellular DNA of all the cells of the organism. Other genes we hope to examine in the near future are human and rat insulin and growth hormones.

Major Findings:  $\alpha$  and  $\beta$  globin RNAs were found in liver and brain tissues of the long-lived young C57BL/6J mouse strain and to increase in relative concentrations with age. A similar change was found for the genes complementary to the c-type virus MuLV cDNA probe. Similar studies were made using the short-lived AKR mouse strain having an unusually high frequency of leukemia. A large age-dependent increase was found for the presence of c-type virus RNA in the thymus gland, but no accompanying expression of the globin genes in either the thymus, brain or liver tissue was found. Studies of the other genes are not yet complete.

Significance to Biomedical Research and the Program of the Institutes: The expression of globin in neurons and adult liver tissue, plus the increase of this expression in older animals, is clearly an unexpected finding and supports the hypothesis that the ability of cells to maintain a differentiated state does decrease with increasing age. Moreover, the age-dependent derepression found for the c-type viruses, which have been indicated as a causative factor in leukemia, suggests that a general decrease in the ability of cells to maintain the repressed state of many other endogenous virus genes may be occurring with increasing age. These results, if confirmed by more extensive studies which are underway, could lead to the identification of a key and perhaps primary aging processes which may underlie many of the mammalian aging



processes.

Proposed Course of Project: A more extensive and detailed study is now being undertaken to further characterize the apparent relation of gene expression of  $\alpha$  and  $\beta$  globin genes and the MuLV c-type virus genes. This will involve investigation of other tissues and different mouse strains (such as AKR). Globin gene expression in human tissues is also being investigated, as well as the insulin and growth hormone genes. It is also of interest to determine if loss of cellular proliferation of in vitro-aged tissue culture cells involves a dedifferentiation process, and so we plan to examine globin gene expression in human cells as a function of passage number.

Our long-term proposed course will be to determine (1) the generality of the relaxed gene expression in terms of tissues and number of different genes effected, (2) if a correlation exists between aging rate of a species and the rate of loss of proper gene control, (3) the molecular mechanisms leading to a loss of gene control and (4) to identify the biological processes that may govern the age-dependent stability of gene expression. These latter two objectives are the objectives of the second and third parts of our research program.

Publications:

Cutler, R.G.: Evolution of longevity in ungulates and carnivores. Gerontology, in press.

Cutler, R.G.: Evolution of human longevity: a critical overview. Mech. Ageing Dev., in press.

Cutler, R.G.: Evolutionary Biology of Senescence. In Behnke, J.A., Finch, C.E. and Moment, G.B. (Eds.): A New Look at Biological Aging. Plenum Press, in press.

Ono, T. and Cutler, R.G.: Age-dependent relaxation of gene repression: Increase of globin and endogenous murine leukemia virus related RNA in brain and liver of mouse. Proc. Natl. Acad. Sci. USA, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00103-02 LCMB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Comparative Mammalian Biochemistry II. Characterization of Chromatin														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
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COOPERATING UNITS (if any)  Laboratory of Nutrition and Endocrinology, NIAMD														
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology														
SECTION Section on Macromolecules														
INSTITUTE AND LOCATION NIH, NIA, Baltimore, Maryland 21224														
TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.6	OTHER: 1.1												
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SUMMARY OF WORK (200 words or less - underline keywords)  <p> <u>Improper</u> gene expression (see Project I) could result from <u>epigenetic</u> and/or <u>mutational-like</u> changes in the <u>genetic</u> apparatus of cells. This project is designed to determine if such <u>physico-chemical</u> alterations of <u>chromatin</u> occur that could account for the altered gene expression previously found. General physico-chemical properties of chromatin from <u>liver</u> and <u>brain</u> of <u>mouse</u> appears to change with age in terms of a <u>decrease in extractability</u> of chromosomal proteins and a decrease in <u>T<sub>3</sub>-hormone binding capacity</u> to chromatin and to intact nuclei. Specific gene changes are also indicated by a decrease in availability of <u>ribosomal genes</u> to <u>DNA-RNA hybridization</u> using ribosomal RNA.         </p>														

GRC/LCMB-202

## Project Description:

Objectives: This project is designed to investigate whether the genetic material of cells remains intact throughout the lifespan of the animal or if significant physico-chemical alterations occur that could lead to the improper function of the cell and possibly to the aging of the organism. The specific objective of these studies is to search for the mechanism at the chromatin level explaining the possible dedifferentiation processes that had been found to occur with increased age in mouse brain and liver tissue.

Methods Employed: The major genetic material of mammalian cells consists of a DNA-protein complex called chromatin. DNA-DNA, DNA-protein and protein-protein complexes of a covalent nature are not normally found in chromatin but could be caused by a number of mutagenic and/or carcinogenic agents as well as many other natural by-products of metabolism that exist in a cell. Although the cell is likely to have repair and protective processes to lower the damage rate of chromatin, the accumulation of such damage could have far-reaching consequences to the proper function of the cell and organism.

We are developing techniques to detect the type and amount of DNA-protein complexes in chromatin as a function of age in C57BL/6J male mice. These are (1) the extractability of chromatin proteins from DNA, (2) the presence and stability of DNA-protein complexes as detected by a nitrocellulose membrane filter assay and (3) the thermal stability of native chromatin complexes.

These techniques are rather crude, however, and could only be expected to detect rather large and non-specific changes. More sensitive and specific methods are therefore being used, such as the hybridization efficiency of the T<sub>3</sub> hormone to chromatin. T<sub>3</sub> hormone is known to bind with high specificity directly to chromatin without first binding to a cytoplasmic receptor protein. Moreover, of additional interest to this method is the report that 6-month post-hypophysectomized rats are rejuvenated in a number of physiological parameters, which might be a result of renewed sensitivity of cells to T<sub>3</sub> hormone stimulation.

Major Findings: (1) Extractability of chromatin proteins decreased with increased age for mouse liver and brain tissues. (2) Ribosomal RNA hybridization efficiency decreases with increased age for mouse liver, brain, kidney, heart and spleen tissues. (3) T<sub>3</sub> hormone binding capacity of chromatin decreases with increased age in liver tissue of rats and remains unchanged in the old 6-month post-hypophysectomized animals.

Significance to Biomedical Research and the Program of the Institute: These experiments suggest that age-dependent alterations do occur in the liver and brain chromatin of mouse and rats. Whether this type of change acts in a causative manner in the aging process by initiating a dedifferentiation process in gene control remains to be determined.

Proposed Course of Project: A more detailed study is now underway to determine the specific biochemical nature of the physico-chemical alterations that

have been found in chromatin. We plan on further developing our assay techniques to increase their sensitivities, to utilize a high-pressure liquid chromatographic system to detect and resolve some of the possible DNA-protein adducts that might exist in the chromatin of older mice and to use specific types of cell populations taken from liver and brain tissues. We also plan to further develop the  $T_3$ -hormone chromatin binding assay to increase its reliability and sensitivity. Through these efforts, we hope to determine what effects the chromatin alterations may have on the transcription activity of a cell and its effect on the ability of cells to maintain their proper differentiated state.

Publications:

Gaubatz, J.W. and Cutler, R.G.: Age-related differences in the number of ribosomal RNA genes of mouse tissues. Gerontology 24: 179-207, 1978.

Cutler, R.G.: Alterations with age in the informational storage and flow systems of mammalian cell. In Bergsma, D. and Harrison, D.E. (Eds.): Genetic Effects on Aging. New York, Alan R. Liss, Inc., 1978, pp. 463-498.

Dean, R.G. and Cutler, R.G.: Absence of significant age-dependent increase of single-stranded DNA extracted from mouse liver nuclei. Exp. Gerontol., in press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00105-01 LCMB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Comparative Mammalian Biochemistry III. Levels of Repair/Protective Processes														
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COOPERATING UNITS (if any)  Department of Neurobiology, Delta Regional Primate Research Center, Tulane University.														
LAB/BRANCH Gerontology Research Center, Laboratory of Cellular and Molecular Biology														
SECTION Section on Macromolecules														
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SUMMARY OF WORK (200 words or less - underline keywords)  The goal of this project is to determine if a correlation exists between levels of <u>potential repair</u> and <u>protective</u> processes and the <u>aging rate</u> of the <u>mammalian</u> species being studied. Currently being studied are <u>superoxide dismutases</u> and <u>selective protein degradation</u> processes. Preliminary results indicate a good correlation of increased level of brain cytoplasmic superoxide dismutase activity with increased lifespan potential in <u>five primate species</u> ranging in lifespan potential from 20 to 100 years. An inverse correlation in these tissues was also found in the level of <u>guanylate cyclase activity</u> . A higher rate of selective degradation of abnormal proteins was found in the longer-lived rodent species, <u>Peromyscus</u> , as compared to <u>Mus</u> .														

GRC/LCMB-205

## Project Description:

Objectives: This project represents a search for genetic repair and protective processes which might act to govern the time-dependent stability of gene expression, and particularly those processes which may have played an important role in the recent evolutionary increase of human longevity. The approach used is to compare levels of genetic repair and protective processes with the innate lifespan potentials of different mammalian species, with emphasis being placed on the primate species.

To date, we have been investigating the selective degradation of abnormal proteins and the levels of superoxide dismutase and guanylate cyclase in both primate and rodent species.

Methods Employed: Selective degradation of altered protein is determined in vivo by measuring the relative rate of degradation of proteins which have incorporated normal vs. amino acid analogues. The major analogue used has been canavanine, an analogue of arginine. Briefly, the technique is to first inject  $^3\text{H}$ -arginine intraperitoneally into a mouse and after about 2 hours to inject  $^{14}\text{C}$ -canavanine. The relative rate of degradation of the two populations of labeled proteins are then determined by measuring the  $^3\text{H}/^{14}\text{C}$  ratio of acid precipitated proteins as a function of time after injection. For this study, we used the wild-type rodent species Mus with a lifespan potential of 3 years and Peromyscus with a lifespan potential of about 8 years.

Superoxide dismutase levels were measured in crude cytoplasmic extractions using a standard assay method. For this study, five different primate species, ranging in lifespan potential from 20 to 100 years, were used.

Major Findings: The longer-lived rodent species, Peromyscus, appears to have about a 2-fold greater capacity in liver to discriminate and degrade the abnormal protein. It is possible, however, that these results might be explained by the degradation rate being a function of the relative amount of amino acid analogue incorporated into the protein. This possibility is currently being investigated.

Levels of superoxide dismutase activity was found to increase in brain tissue with increased lifespan potential of the primate species by a factor of about 3-fold. An inverse function in the level of guanylate cyclase was found in the same species.

Significance to Biomedical Research and the Program of the Institutes: These studies support the concept that an increase in the level of activity of a common set of repair and protective processes in the mammalian species may play an important role in governing their different aging rates. It is also possible that the processes being studied could act to protect the genetic apparatus and thus be important in stabilizing the differentiated state of the cell.

Proposed Course of Project: Controls testing the dependence of degradation

rate on the level of incorporation of amino acid analogue are now being conducted. Superoxide dismutase levels will be measured in additional primate species and in other tissues. We plan to extend these investigations in the near future to include catalase, glutathione peroxidase, vitamin C, vitamin E, cAMP, cGMP, adenylate cyclase, and the depurination DNA repair levels in rodent and primate species.

Publications:

None.

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR AGING  
NATIONAL INSTITUTE ON AGING

Studies of the clinical, behavioral, and biological aspects of aging have, over the last thirty years, provided the necessary conceptual basis for departure into the biochemistry and biophysics responsible for these time-dependent phenomena. Accordingly, the Laboratory of Molecular Aging conducts biochemical and biophysical research aimed to: (1) determine the mechanisms by which physiological processes are altered in the aged; and (2) use this new information as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. Our research this year continues to focus on the critical area of physiological control systems which undergo perturbations leading to the inability of the aged to maintain homeostasis. These investigations impact on the fundamental mechanisms of age-dependent changes in (a) renal function, (b) cardiac function, (c) cerebral function, (d) smooth muscle activity, (e) metabolism, and (f) on mechanisms basic to the development of aging hypothesis which transcend specific physiological systems. With improved methods and new technologies for studying basic biological phenomena, the biochemical and biophysical solutions of questions on aging are an attainable goal.

In the aged, the maintenance of blood pH and the homeostatic mechanism to respond to a state of acidosis by secretion of acid is less competent. Since ammonia production is a major mechanism whereby the kidney produces acid which can be excreted in response to the state of acidosis, a study was initiated to examine the question whether the physiological impairment seen in senescence reflected a biochemical decrement at the level of mitochondrial ammonia genesis and glutamine metabolism. Rats, 6 months and 24 months, were made acidotic by providing 1.5%  $\text{NH}_4\text{Cl}$  solutions as the sole source of drinking water for seven days. It was found that kidney mitochondria from both 6 and 24 month rats showed adaptation to the state of acidosis, with the adaptation being significantly less in mitochondria from the old animals. Thus, values for rates of  $\text{NH}_3$  production, flux through the enzyme glutaminase and flux through glutamate dehydrogenase were  $22.9 \pm 0.9$  ( $21.5 \pm 1.0$ ),  $15.0$  ( $14.5$ ) and  $7.9 \pm 0.2$  ( $7.0 \pm 0.4$ ) nmol/min/mg of mitochondrial protein, respectively, with data from 24 month animals in parentheses. Thus, in the unstressed animals the mitochondria did not differ. In the acidotically stressed animals the corresponding values from 6 month and 24 month old rats were  $38.0 \pm 1.8$  ( $29.6 \pm 2.7$ ),  $23.2$  ( $18.8$ ) and  $14.8 \pm 0.8$  ( $10.8 \pm 1.2$ ). These findings demonstrate that flux through glutaminase and glutamate dehydrogenase, the two reactions responsible for the production of  $\text{NH}_3$  increases significantly in response to acidosis and that the degree of adaptation is significantly less in the old animal. Preliminary observations suggest that far less mitochondrial protein is recovered per kidney or per gram of kidney tissue in 24 month rats relative to 6 month rats and it is considered highly likely that a diminished content of mitochondria in the kidneys of the old animals would be a major contributory factor in limiting  $\text{NH}_3$  production by the kidney in response to acidosis.

Isolated kidney mitochondria were also characterized with respect to their ability to oxidize other substrates. Mitochondria from 24 month old rats



The relationship between age-associated decrements in striatal dopamine levels plus amphetamine-induced rotational behavior and the activities of tyrosine-hydroxylase, DOPA-decarboxylase, dopamine-stimulated adenylate cyclase, and cyclic nucleotide phosphodiesterase was examined in collaboration with investigators of the Laboratory of Behavioral Sciences. A 15% decrease in tyrosine hydroxylase was found in senescent rats. The subcellular distribution of the enzyme was not altered. Preliminary results indicate little or no change in DOPA-decarboxylase activity or in the kinetic properties of the enzyme. Results, to date, suggest a decrease in both sensitivity and responsiveness of adenylate cyclase to dopamine in 24 vs 6 month old rats. No significant age-changes were found in phosphodiesterase activity.

Studies were continued on the biochemical and biophysical mechanisms of renal membrane transport systems to define how age-dependent changes in membrane properties are reflected in decrements in renal function and in a decreased capacity in the aged to compensate for life-threatening disturbances in fluid and solute homeostasis. Last year, we reported that the rate of D-glucose uptake in brush border membrane vesicles was correlated with the  $\text{Na}^+$  electrochemical potential; each component, the electrical or chemical gradient, when assayed independently, supported the uphill transport of the sugar. These findings were consistent with the  $\text{Na}^+$  gradient hypothesis, which postulates that the non-electrolyte is symported with  $\text{Na}^+$  and the  $\text{Na}^+$  electrochemical gradient across the luminal membrane in intact systems provides the energy for the uphill transport of the sugar. Because the brush border membrane potential was shown to be inside negative, the question arose as to whether the electrogenic cotransport of  $\text{Na}^+$  and D-glucose would decrease the potential. Since electrophysiological techniques to monitor potentials in isolated microvillar membranes were precluded, optical probes which assessed changes in potential were sought. The fluorescent dye, 3,3'-dipropylthiodicarbocyanine iodine [ $\text{DiS-C}_3\text{-(5)}$ ] proved to be an effective monitor of membrane potentials in this system. Thus, by establishing valinomycin-induced  $\text{K}^+$ -diffusion potentials in isolated brush border membrane vesicles it was shown that an increase in fluorescence was indicative of membrane depolarization (interior became more positive) and that a decrease in fluorescence was indicative of hyperpolarization of the membrane (interior became more negative). When D-glucose was added to a suspension of renal brush border membrane vesicles equilibrated in a  $\text{Na}^+$ -containing medium there was a rapid transient increase in the fluorescence of the probe,  $\text{DiS-C}_3\text{-(5)}$ . This sugar-induced response was stereospecific for the D-isomer, dependent on  $\text{Na}^+$ , inhibited by phlorizin, and blocked by ionophores, valinomycin + nigericin, which dissipate ionic gradients. The enhancement in fluorescence suggests the entrance into the vesicle of  $\text{Na}^+$ , cotransported with the sugar. This would lead to the interior of the membrane vesicle becoming more positive, resulting in depolarization of the membrane potential. That the sugar induced the transport of  $\text{Na}^+$  was confirmed by direct measurement of  $^{22}\text{Na}$  uptake. Therefore, the  $\text{Na}^+$ -sugar cotransport system provides a mechanism for D-glucose to enhance the flux of  $\text{Na}^+$  as well as for the  $\text{Na}^+$  electrochemical gradient to stimulate the transport of D-glucose.

D-Glucose-dependent  $\text{Na}^+$  uptake was studied in more detail. Our previous work led to certain predictions for  $\text{Na}^+$  transport stimulated by sugar. These predictions were: (1) D-glucose would stimulate  $\text{Na}^+$  uptake but L-glucose

would not. (2) D-glucose would stimulate  $\text{Na}^+$  uptake but not the uptakes of  $\text{K}^+$  or  $\text{Rb}^+$ . (3)  $\text{Na}^+$  uptake would be dependent on both the  $\text{Na}^+$  and D-glucose concentrations. (4) Ionophores, such as gramicidin, nystatin, or nigericin + valinomycin should dissipate  $\text{Na}^+$  uptake stimulated by D-glucose. (5) Internal negative potentials should stimulate D-glucose-dependent  $\text{Na}^+$  uptake. (6) Phlorizin should inhibit D-glucose-dependent  $\text{Na}^+$  uptake. All these predictions were confirmed by our investigation of D-glucose-dependent  $\text{Na}^+$  uptake.

The stoichiometry of the  $\text{Na}^+$  transported per D-glucose molecule taken up is one aspect of the  $\text{Na}^+$ -D-glucose cotransport system which has not been examined directly. Studies on the accumulation of D-glucose as a function of various  $\text{Na}^+$  gradients, in the absence of a membrane potential, suggests a 1:1 stoichiometry between sugar and  $\text{Na}^+$  uptake.

The relatively small yield of brush border membranes with each preparation severely hampers efforts to purify sugar and amino acid carriers, since the only assay for the carrier in membrane reconstitution studies is transport function, itself, rather than catalytic activity as with cation transporting ATPases. Thus, a procedure for preserving transport function of these membranes is critically needed. It was found that transport of D-glucose and L-proline in renal brush border membrane vesicles was preserved by storage of the membranes at low temperature in 20% glycerol. Uptake of the sugar by the stored membranes represented transport into an intravesicular space, rather than binding, and was  $\text{Na}^+$  gradient-dependent, concentrative, stereospecific, and inhibited by phlorizin. Uptake of L-proline by the stabilized membranes was  $\text{Na}^+$  gradient-dependent and uphill. Preservation of the sugar and amino acid transport properties by this technique will facilitate studies which require that the physiological functions of the membranes be retained.

The mechanism of the transport of L-glutamate by renal brush border membrane vesicles was investigated. This was of great interest since glutamate bears a negative charge at neutral pH, in contrast to the net neutrally charged compounds L-proline, L-alanine, and D-glucose. Hence, glutamate might be expected to exhibit transport properties different from the others, which were found to be transported in an electrogenic fashion. The imposition of a large  $\text{Na}^+$  gradient (medium > vesicle) resulted in a marked increase in the initial rate of uptake and in the transient accumulation of glutamate against its concentration gradient, with an "offshoot" similar to that seen with glucose, proline and alanine. Uphill transport was specifically energized by  $\text{Na}^+$  and the initial rate of uptake of L-glutamate was dependent on the concentration of extravesicular  $\text{Na}^+$ . Accelerated exchange diffusion was demonstrated only with L-glutamate and not with the D-isomer. Moreover, the glutamate carrier recognized L-aspartate and only a few derivatives of L-glutamate. Other decarboxylic acids, neutral or basic amino acids, and imino acids were not recognized by the L-glutamate carrier.  $\text{Na}^+$ -dependent L-glutamate transport, in contrast to the electrogenic transports of D-glucose, L-proline, and L-alanine, appeared to be an electroneutral process by the following criteria: (1) L-glutamate transport was not affected by impermeant or permeant anions. (2) L-glutamate uptake was not affected by valinomycin-induced membrane potentials, either interior positive or interior negative. (3) The cotransport of  $\text{Na}^+$ -L-glutamate was not associated with a transient decrease in membrane potential as monitored by the fluorescent probe DiS-C<sub>3</sub>-(5).

The  $\text{Na}^+$  gradient-dependent uphill transport of L-glutamate was augmented additionally by the imposition of a  $\text{K}^+$  gradient (vesicle  $>$  medium) directed opposite to the  $\text{Na}^+$  gradient. This effect was specific for  $\text{K}^+$ ;  $\text{Li}^+$  and choline and tetraethylammonium ions were inactive, while  $\text{Rb}^+$  could substitute for  $\text{K}^+$ . The magnitude of the L-glutamate "overshoot" found in the presence of both (internal)  $\text{K}^+$  and (external)  $\text{Na}^+$  gradients was not affected by the anion species. Addition of the ionophore nigericin severely inhibited the uptake of L-glutamate observed in the presence of both the  $\text{Na}^+$  and  $\text{K}^+$  gradients, presumably because this ionophore catalyzed the electroneutral exchange of  $\text{Na}^+$  for  $\text{K}^+$  and, thus, effected the collapse of both gradients. The mechanism underlying this unique observation, which was not seen for the transports of sugars and other amino acids, will be the subject of further study.

An investigation of the transport of phosphate by renal brush border membrane vesicles was initiated. Uptake represented the transport of the anion across the membrane into the intravesicular space. Phosphate uptake was  $\text{Na}^+$  gradient-dependent. The initial rate of phosphate transport with the  $\text{Na}^+$  gradient was 20- to 50-times greater than in the absence of the gradient. Uptake was inhibited by arsenate. Transport of phosphate increased with increases in pH; uptake at pH 8.5 was 4-times greater than at pH 6.0. This finding suggests that the divalent phosphate anion,  $\text{HPO}_4^{2-}$ , was the species preferentially transported; however, further studies are required to confirm this tentative suggestion. Modification of the membrane potential across the brush border membrane by the use of sodium salts comprised of anions of different modes of permeability and specific ionophores to generate large  $\text{K}^+$  diffusion potentials indicates that phosphate uptake was an electroneutral process. Ionophores that enhanced membrane conduction for  $\text{Na}^+$ , either electro-neutrally or electrogenically, completely blocked concentrative transport.

Studies on the regulation of phosphate transport were initiated. Two control systems were considered: (1) diet; and (2) regulation by the hormone, parathyroid hormone. The initial rate of phosphate uptake by membrane preparations from animals on a low phosphate diet was about twice that of animals on a high phosphate diet. Preliminary studies suggest that parathyroid hormone, mediated via cyclic nucleotide-dependent systems, altered the uptake of phosphate in membranes isolated subsequent to hormonal treatment.

The control of various physiological systems as well as age-related alterations in these vital functions were previously reported to be regulated by hormones, putative neurotransmitters, and drugs whose modes of action were mediated via the cyclic nucleotides, adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP). Thus, studies on the enzymes that determine tissue levels of the cyclic nucleotides, *e.g.* adenylate cyclase, guanylate cyclase, and phosphodiesterases, and enzymes, *e.g.* protein kinases, whose activities are regulated by the cyclic nucleotides, are of great significance.

Parathyroid hormone and prostaglandin  $\text{E}_1$  receptors and adenylate cyclase sensitive to these hormones were localized on the basal-lateral membrane of the renal cortical tubule. GMP-PNP, and more effectively, GTP, markedly enhanced the stimulation of adenylate cyclase by these hormones. For example, parathyroid hormone (20 U/ml) increased adenylate cyclase 200% relative to



the basal activity. With the addition of 0.1  $\mu$ M GTP, which affected only a 10 to 20% activation of the enzyme by itself, parathyroid hormone now stimulated adenylate cyclase over 400%. The actions of GTP were specific, since the nucleotide did not stimulate the activation of adenylate cyclase by epinephrine nor glucagon. The presence of cytosolic protein factors which regulated hormonal activation of adenylate cyclase was also found and these are being isolated and their actions characterized.

Regulation of the synthesis of cyclic GMP was examined in considerable detail. Noteworthy were the interactions of divalent cations,  $Mn^{2+}$ ,  $Mg^{2+}$ , and  $Ca^{2+}$ , with the cytosolic guanylate cyclase from different tissues. Guanylate cyclase activities of the kidney, liver, and lung were strongly dependent on  $Mn^{2+}$ . In contrast, the enzyme in smooth muscle of the colon, aorta, and vas deferens was active with  $Mg^{2+}$  as well as with  $Mn^{2+}$ .  $Ca^{2+}$  was ineffective in all tissues. Preincubation of colon extracts, but not those of kidney and liver, increased guanylate cyclase activity. The  $Mg^{2+}$ -dependent activity was preferentially enhanced by this treatment. These results suggest that when the enzyme was autoactivated by endogenous factors it became more  $Mg^{2+}$ -dependent. Dithiothreitol strongly inhibited the  $Mg^{2+}$ -dependent colon enzyme, whereas activities in kidney and liver were not affected and the response of the enzyme in lung was intermediate. This suggests that autoactivation involved an oxidative-reductive alteration of the enzyme.  $Ca^{2+}$  markedly inhibited the  $Mg^{2+}$ -dependent activity in smooth muscles but  $Mg^{2+}$ -dependent activities in lung, liver, and kidney were not influenced appreciably. Exogenous activators, dehydroascorbate and  $NaN_3$ , increased guanylate cyclase, assayed with either  $Mg^{2+}$  or  $Mn^{2+}$ . However, the relative stimulation of the enzyme assayed with  $Mg^{2+}$  was greater than with  $Mn^{2+}$ . When activated by these exogenous agents, guanylate cyclase in all tissues became inhibitable by  $Ca^{2+}$ . These findings suggest that guanylate cyclase in smooth muscles was in a partially activated form.

The endogenous activating factors in colon smooth muscle were heat-stable, largely extractable with chloroform:methanol, and cochromatographed with authentic fatty acids. Arachidonic acid stimulated colon guanylate cyclase and enhancement of the  $Mg^{2+}$ -dependent activity was blocked by  $Ca^{2+}$ . This strongly infers that a significant part of the endogenous activating factors in the colon was fatty acids or their derivatives. The colon activators had only minimal effects on the enzyme in the kidney. Similarly prepared activator extracts from the kidney increased colon guanylate cyclase but did not stimulate the renal enzyme. Thus, the ability of the enzyme to be stimulated by endogenous activators was dependent on the tissue from which the enzyme was derived.

These findings strongly support the possibility that the interactions of  $Mg^{2+}$  and  $Ca^{2+}$  on guanylate cyclase in smooth muscles were of regulatory significance to the contractile response of the muscle. It is proposed as a working hypothesis that cyclic GMP and  $Ca^{2+}$  might participate in reciprocal negative feedback mechanisms.

In the course of the above studies on guanylate cyclase in smooth muscle, it was found that colon guanylate cyclase activity was inhibited by the addition of the cytosolic fraction of kidney or liver. This observation suggested



that these two latter tissues contained a factor which inhibited the activity of guanylate cyclase. This factor is being purified and its mode of action is being characterized.

The permeability properties of membranes are presumed to be regulated by phosphorylation/dephosphorylation of specific membrane proteins, reactions catalyzed by cyclic AMP-dependent and -independent protein kinases and phosphatases. This year we continued to characterize these enzymes in the renal cortex. Protein kinases were localized in both the cytosol and brush border membrane. The cytosol contained two distinct cyclic AMP-dependent and three cyclic AMP-independent kinases, as judged by separation on DEAE-cellulose in cyclic AMP containing buffer. The cyclic AMP-dependent kinases had greater activity with histone F2b as protein substrate than with protamine, whereas the independent forms were more specific for protamine. Salt and detergent extraction of the brush border membrane revealed the presence of two cyclic AMP-dependent and two cyclic AMP-independent enzymes. Kinetic analysis of the cytosolic cyclic AMP-dependent enzymes showed no differences with respect to  $K_a$  for cyclic AMP,  $K_m$  for ATP, or substrate specificity. They did differ with respect to dissociation by salt and histone, and thus were classified as type I and type II (predominantly type II). Both membrane cAMP-dependent enzymes fit the criteria of a type II kinase. Of particular interest was the finding that the membrane protein kinases differed with respect to their ease of extraction from the membrane. High salt concentrations extracted one of the cyclic AMP-dependent and one of the -independent enzymes. The other two kinases, one cyclic AMP-dependent and the other -independent, required detergent for extraction. These findings suggest that the protein kinases are oriented specifically in the membrane, either as intrinsic or extrinsic proteins.

The mechanism by which phenothiazines inhibit a partially purified cerebral cortical phosphodiesterase having a high stimulatory response to a  $Ca^{2+}$ -dependent regulatory (CDR) protein and specificity towards guanosine 3',5'-(cyclic) monophosphate was studied. Trifluoperazine (50  $\mu$ M) completely blocked the  $Ca^{2+}$ ·CDR-stimulated activity but had no effect on the basal activity. The magnitude of the inhibition depended on the concentrations of both inhibitor and activator. With increasing concentration of CDR, the enzyme became less sensitive to the phenothiazine. In kinetic analyses of the trifluoperazine-CDR interaction, plots of  $1/V$  vs  $1/CDR$  and  $1/V$  vs trifluoperazine concentration were non-linear with upward curvature. Phenothiazine inhibition was enhanced by decreasing the pH of the reaction mixture from 8.0 to 6.8, a finding which correlated with a previously reported increase in binding of the antipsychotic drug to  $Ca^{2+}$ ·CDR when pH was lowered. These results argue against simple competition between inhibitor and  $Ca^{2+}$ ·CDR at the activator binding site on the phosphodiesterase. Instead, they suggest that phenothiazines, upon binding to  $Ca^{2+}$ ·CDR, render the latter ineffective in stimulating the enzyme, thus depleting the level of active CDR and inhibiting the activator-dependent phosphodiesterase. It is proposed that analogous phenothiazine- $Ca^{2+}$ ·CDR interactions may be involved in the mode of action of the drug on the activities of other  $Ca^{2+}$ ·CDR-dependent enzymes.

A study was initiated to describe the molecular organization and orientation of the major membrane constituents, using the renal brush border membrane as

a model. Our attention was focused on the membrane glycoproteins, especially those containing sialic acid as the terminal residue. Electrophoresis of the brush border membrane on SDS gels revealed that four of the protein bands stained positively with PAS, indicative of glycoproteins. The membranes contained about 5  $\mu$ g of sialic acid per mg of membrane protein. Treatment with neuraminadase removed 50-70% of the total sialic acid. This removal of sialic acid did not markedly alter the  $\text{Na}^+$ -dependent transports of D-glucose nor L-proline, suggesting that sialic acid was not essential for transport activity. However, a small but consistent difference between sialylated and desialylated membranes was observed when the kinetics of D-glucose uptake were carefully examined. Since in the absence of the  $\text{Na}^+$  gradient the removal of sialic acid did not alter the pattern of sugar uptake, these findings suggest that the D-glucose carrier was not directly affected but that the maintenance of the  $\text{Na}^+$  electrochemical gradient was altered by the removal of sialic acid.

NAD-linked isocitrate dehydrogenase is rate-limiting in the operation of the Krebs cycle in mitochondria. A detailed kinetic analysis was carried out on the effects of various activators and inhibitors of the enzyme, in the presence of divalent cations and chelators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 AG 00041-05 LMA
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Physiological Control Systems and Aging I Membrane Transport Mechanisms		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  OTHER:	B. Sacktor L. Cheng S. Hilden L. Noronha-Blob E. Schneider	Chief, Lab. Molec. Aging Staff Fellow (EOD 11/6/77) Staff Fellow Staff Fellow (EOD 1/29/78) Staff Fellow    LMA NIA LMA NIA LMA NIA LMA NIA LMA NIA
COOPERATING UNITS (if any) None		
LAB/BRANCH Gerontology Research Center, Laboratory of Molecular Aging		
SECTION Intermediary Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS: 6.5	PROFESSIONAL: 4.0	OTHER: 2.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) This study of <u>membrane transport</u> is targeted to provide the basic scientific information needed to: determine the mechanisms by which <u>physiological control systems</u> are altered in the aged; and use this new information as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. <u>Membrane vesicles</u> derived from the luminal <u>brush border</u> segment and the antiluminal <u>basal-lateral</u> region of the <u>renal tubule epithelial cell plasma membrane</u> are used as model systems. Topics investigated include: 1) the role of the <u>membrane potential</u> in the $\text{Na}^+$ <u>gradient-dependent uptake</u> of D-glucose by brush border membrane vesicles; 2) the mechanisms and specificities of <u>amino acid transport</u> systems; 3) the mechanism and regulation of <u>phosphate transport</u> ; 4) mechanism of the D-glucose dependent $\text{Na}^+$ <u>transport</u> ; 5) the isolation and characterization of the $\text{Na}^+$ <u>gradient-dependent glucose carrier</u> by reconstitution of <u>synthetic membranes</u> ; 6) molecular <u>organization and orientation of membrane</u> components in the membrane.		

GRC/LMA-215



## Project Description:

Objectives: These studies are targeted to define the biochemical and biophysical mechanisms whereby age-dependent changes in membrane transport perturb physiological control systems which lead to a failure to maintain homeostasis. The investigations impact on vital functions, including those in the kidney, brain, heart, gastrointestinal and urogenital tracts. For example, a typical experimental question may be: Why does the kidney of the aged have a decreased capacity to compensate for life-threatening disturbances in fluid and solute homeostasis? The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) role in selective vectorial transport; (3) hormonal regulation of function; (4) catalytic function; (5) turnover; and (6) failure to maintain structure, leading to cell death.

Methods Employed: Membrane vesicles derived from the luminal brush border segment and the antiluminal basal-lateral region of the renal tubule epithelial cell plasma membrane are used as model systems. Components of the membrane are resolved and reconstituted to determine whether defects are due to changes in a specific constituent or in the membrane milieu.

Major Findings: Studies were continued on the biochemical and biophysical mechanisms of renal membrane transport systems. Last year, we reported that the rate of D-glucose uptake in brush border membrane vesicles was correlated with the  $\text{Na}^+$  electrochemical potential; each component, the electrical or chemical gradient, when assayed independently, supported the uphill transport of the sugar. These findings were consistent with the  $\text{Na}^+$  gradient hypothesis, which postulates that the non-electrolyte is symported with  $\text{Na}^+$  and the  $\text{Na}^+$  electrochemical gradient across the luminal membrane in intact systems provides the energy for the uphill transport of the sugar. Because the brush border membrane potential was shown to be inside negative, the question arose as to whether the electrogenic cotransport of  $\text{Na}^+$  and D-glucose would decrease the potential. Since electrophysiological techniques to monitor potentials in isolated microvillar membranes were precluded, optical probes which assessed changes in potential were sought. The fluorescent dye, 3,3'-dipropylthiodi-carbocyanine iodine [ $\text{DiS-C}_3\text{-(5)}$ ] proved to be an effective monitor of membrane potentials in this system. Thus, by establishing valinomycin-induced  $\text{K}^+$ -diffusion potentials in isolated brush border membrane vesicles it was shown that an increase in fluorescence was indicative of membrane depolarization (interior became more positive) and that a decrease in fluorescence was indicative of hyperpolarization of the membrane (interior became more negative). When D-glucose was added to a suspension of renal brush border membrane vesicles equilibrated in a  $\text{Na}^+$ -containing medium there was a rapid transient increase in the fluorescence of the probe,  $\text{DiS-C}_3\text{-(5)}$ . This sugar-induced response was stereospecific for the D-isomer, dependent on  $\text{Na}^+$ , inhibited by phlorizin, and blocked by ionophores, valinomycin + nigericin, which dissipate ionic gradients. The enhancement in fluorescence suggests the entrance into the vesicle of  $\text{Na}^+$ , cotransported with the sugar. This would lead to the interior of the membrane vesicle becoming more positive, resulting in depolarization of the membrane potential. That the sugar induced the transport of



$\text{Na}^+$  was confirmed by direct measurement of  $^{22}\text{Na}^+$  uptake. Therefore, the  $\text{Na}^+$ -sugar cotransport system provides a mechanism for D-glucose to enhance the flux of  $\text{Na}^+$  as well as for the  $\text{Na}^+$  electrochemical gradient to stimulate the transport of D-glucose.

The stoichiometry of the  $\text{Na}^+$  transported per D-glucose molecule taken up is one aspect of the  $\text{Na}^+$ -D-glucose cotransport system which has not been examined directly. Studies on the accumulation of D-glucose as a function of various  $\text{Na}^+$  gradients, in the absence of a membrane potential, suggests a 1:1 stoichiometry between sugar and  $\text{Na}^+$  uptake.

It was reported by others that brush border membrane vesicles from rat intestine exhibited slightly elevated levels of  $\text{Na}^+$ -dependent glucose transport in alloxan-induced diabetic as compared to normal animals. These workers speculated that membranes from these diabetic animals had a decreased conductance to  $\text{Na}^+$ , resulting in an increased and prolonged  $\text{Na}^+$  electrochemical gradient. In an attempt to confirm this hypothesis and to determine whether this effect of the diabetic state on transport could be reversed by the administration of insulin we induced a very severe diabetic state in rats with streptozotocin, as evident by loss of weight and excretion of glucose in the urine. Upon comparing glucose transport in brush border membrane vesicles from intestine and from kidney, we found very little, if any, difference between normal and diabetic rats. Thus, the reported observation was not confirmed by our experimental conditions.

The mechanism of the transport of L-glutamate by renal brush border membrane vesicles was investigated. This was of great interest since glutamate bears a negative charge at neutral pH, in contrast to the net neutrally charged compounds L-proline, L-alanine, and D-glucose. Hence, glutamate might be expected to exhibit transport properties different from the others, which were found to be transported in an electrogenic fashion. The imposition of a large  $\text{Na}^+$  gradient (medium > vesicle) resulted in a marked increase in the initial rate of uptake and in the transient accumulation of glutamate against its concentration gradient, with an "offshoot" similar to that seen with glucose, proline and alanine. Uphill transport was specifically energized by  $\text{Na}^+$  and the initial rate of uptake of L-glutamate was dependent on the concentration of extravesicular  $\text{Na}^+$ . Accelerated exchange diffusion was demonstrated only with L-glutamate and not with the D-isomer. Moreover, the glutamate carrier recognized L-aspartate and only a few derivatives of L-glutamate. Other decarboxylic acids, neutral or basic amino acids, and imino acids were not recognized by the L-glutamate carrier.  $\text{Na}^+$ -dependent L-glutamate transport, in contrast to the electrogenic transports of D-glucose, L-proline, and L-alanine, appeared to be an electroneutral process by the following criteria: (1) L-glutamate transport was not affected by impermeant or permeant anions. (2) L-glutamate uptake was not affected by valinomycin-induced membrane potentials, either interior positive or interior negative. (3) The cotransport of  $\text{Na}^+$ -L-glutamate was not associated with a transient decrease in membrane potential as monitored by the fluorescent probe DiS-C<sub>3</sub>-(5).

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An investigation of the transport of phosphate by renal brush border membrane vesicles was initiated. Uptake represented the transport of the anion across the membrane into the intravesicular space. Phosphate uptake was  $\text{Na}^+$  gradient-dependent. The initial rate of phosphate transport with the  $\text{Na}^+$  gradient was 20- to 50-times greater than in the absence of the gradient. Uptake was inhibited by arsenate. Transport of phosphate increased with increases in pH; uptake at pH 8.5 was 4-times greater than at pH 6.0. This finding suggests that the divalent phosphate anion,  $\text{HPO}_4^{2-}$ , was the species preferentially transported; however, further studies are required to confirm this tentative suggestion. Modification of the membrane potential across the brush border membrane by the use of sodium salts comprised of anions of different modes of permeability and specific ionophores to generate large  $\text{K}^+$  diffusion potentials indicates that phosphate uptake was an electroneutral process. Ionophores that enhanced membrane conduction for  $\text{Na}^+$ , either electro-neutrally or electrogenically, completely blocked concentrative transport.

$\text{Li}^+$  was shown previously to be slightly effective as a  $\text{Na}^+$  substitute in at least some  $\text{Na}^+$ -dependent transport systems in the brush border membrane. Since it has been suggested that  $\text{Li}^+$  may have its therapeutic effects via  $\text{Na}^+$  transport, brush border membranes may be an excellent model system to study the mode of action of this agent because of the variety of  $\text{Na}^+$ -dependent transport systems present in the same preparation (electrogenic and electro-neutral; sugars and amino acids; symports and antiports, etc.). Preliminary studies revealed that low  $\text{Li}^+$  levels in the presence of  $\text{Na}^+$  affected several transport systems in different ways (stimulation, inhibition or no effect).

The relatively small yield of brush border membranes with each preparation severely hampers efforts to purify sugar and amino acid carriers, since the only assay for the carrier in membrane reconstitution studies is transport function, itself, rather than catalytic activity as with cation transporting ATPases. Thus, a procedure for preserving transport function of these membranes is critically needed. It was found that transport of D-glucose and L-proline in renal brush border membrane vesicles was preserved by storage of the membranes at low temperature in 20% glycerol. Uptake of the sugar by the stored membranes represented transport into an intravesicular space, rather than binding, and was  $\text{Na}^+$  gradient-dependent, concentrative, stereospecific, and inhibited by phlorizin. Uptake of L-proline by the stabilized membranes

was  $\text{Na}^+$  gradient-dependent and uphill. Preservation of the sugar and amino acid transport properties by this technique will facilitate studies which require that the physiological functions of the membranes be retained.

D-Glucose-dependent  $\text{Na}^+$  uptake was studied in more detail. Our previous work led to certain predictions for  $\text{Na}^+$  transport stimulated by sugar. These predictions were: (1) D-Glucose would stimulate  $\text{Na}^+$  uptake but L-glucose would not. (2) D-Glucose would stimulate  $\text{Na}^+$  uptake but not the uptakes of  $\text{K}^+$  or  $\text{Rb}^+$ . (3)  $\text{Na}^+$  uptake would be dependent on both the  $\text{Na}^+$  and D-glucose concentrations. (4) Ionophores such as gramicidin, nystatin, or nigericin + valinomycin should dissipate  $\text{Na}^+$  uptake stimulated by D-glucose. (5) Internal negative potentials should stimulate D-glucose-dependent  $\text{Na}^+$  uptake. (6) Phlorizin should inhibit D-glucose-dependent  $\text{Na}^+$  uptake. All these predictions were confirmed by our investigation of D-glucose-dependent  $\text{Na}^+$  uptake.

A study was initiated to describe the molecular organization and orientation of the major membrane constituents, using the renal brush border membrane as a model. Our attention was focused on the membrane glycoproteins, especially those containing sialic acid as the terminal residue. Electrophoresis of the brush border membrane on SDS gels revealed that four of the protein bands stained positively with PAS, indicative of glycoproteins. The membranes contained about 5  $\mu\text{g}$  of sialic acid per mg of membrane protein. Treatment with neuraminadase removed 50-70% of the total sialic acid. This removal of sialic acid did not markedly alter the  $\text{Na}^+$ -dependent transports of D-glucose nor L-proline, suggesting that sialic acid was not essential for transport activity. However, a small but consistent difference between sialylated and desialylated membranes was observed when the kinetics of D-glucose uptake were carefully examined. Since in the absence of the  $\text{Na}^+$  gradient the removal of sialic acid did not alter the pattern of sugar uptake, these findings suggest that the D-glucose carrier was not directly affected but that the maintenance of the  $\text{Na}^+$  electrochemical gradient was altered by the removal of sialic acid.

#### Significance to Biomedical Research and to the Program of the Institute:

These studies, using renal plasma membrane vesicles as model membranes to investigate transport processes, describe mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

#### Proposed Course of the Project: Studies on the mechanisms of transport will:

(1) further probe the interrelations between membrane potential and active solute transport, (2) examine the mechanism of amino acid transport in renal membrane vesicles, (3) determine the specificity of amino acid transport in the brush border and basal lateral membranes in the intestinal epithelial cell, (4) describe the mechanism of phosphate transport and its regulation, (5) examine the mechanism of sulfate transport, (6) examine  $\text{Na}^+ - \text{H}^+$  exchange systems as a mechanism of acid-base and salt-water balance, (7) explore the recent



technique of membrane resolution-reconstitution to define transport systems in terms of molecular organization of the membrane, and (8) continue to apply these fundamental studies to transport systems in the kidney of the aged dog.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00042-05 LMA

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging II  
Mechanisms of Hormonal Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Sacktor	Chief, Lab. Molec. Aging	LMA NIA
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	J. Froehlich	Medical Officer	LMA NIA
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COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

St

Intermediary Metabolism and Biophysics Sections

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:

7.3

PROFESSIONAL:

5.0

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of hormonal regulation is targeted to define the biochemical mechanisms whereby age-dependent changes perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. Investigations are focused on the biochemical interactions of hormones, which are mediated via cyclic AMP and cyclic GMP. Topics investigated include: 1) hormone receptors in membranes; 2) adenylate cyclase and guanylate cyclase activities; 3) phosphorylation of membrane proteins by cyclic nucleotide-dependent and -independent protein kinases; 4) control of cyclic nucleotide levels by regulation of phosphodiesterase activities. Hormonal regulatory systems in kidney, heart, brain, colon, aorta, and ductus deferens were studied.

GRC/LMA-221

## Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in hormonal regulation perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP. The research has impact on the mechanism underlying age-related changes in renal, neural, cardiac and smooth muscle function.

Methods Employed: Membrane and cytosol preparations derived from kidney, brain, heart, aorta, colon and ductus deferens were used.

Major Findings: The increase in cardiac contractility associated with stress is postulated to involve increased release of  $\text{Ca}^{2+}$  to the myofilaments from the sarcoplasmic reticulum secondarily to increased  $\text{Ca}^{2+}$  accumulation by the sarcoplasmic reticulum. The latter is triggered by a cAMP-dependent mechanism that involves phosphorylation of a specific membrane protein by protein kinase. Because previous work in this laboratory found that  $\text{Ca}^{2+}$  transport was decreased in sarcoplasmic reticulum prepared from old hearts, the question arose whether hormonally regulated  $\text{Ca}^{2+}$  transport was able to compensate for this loss of activity or whether it too diminished with age. Examination of this problem was begun with an attempt to define the conditions necessary for maximizing the cAMP-dependent response in sarcoplasmic reticulum prepared from young and old rat hearts. It was found that oxalate-dependent  $\text{Ca}^{2+}$  accumulating activity was stimulated by cAMP at concentrations ranging between  $10^{-9}$  and  $10^{-5}$  M in the presence of added protein kinase.  $\text{Ca}^{2+}$  concentrations that elicited less than maximal transport velocities in the absence of cAMP gave the largest increase in activity in response to cAMP. Preliminary data on membranes isolated from 12 and 24 month old rat hearts indicate a maximum response to cAMP of 30-40%, suggesting that cAMP in sarcoplasmic reticulum from old hearts was able to increase  $\text{Ca}^{2+}$  transport activity to baseline levels seen in the sarcoplasmic reticulum from young hearts. This functional response of the sarcoplasmic reticulum to cyclic AMP and protein kinase infers phosphorylation of sarcoplasmic reticulum membrane protein. Conditions for kinase stimulated phosphorylation are being optimized in order to compare levels of phosphorylation of specific proteins in membranes from young and old hearts.

In collaborative studies with investigators from the Clinical Physiology Branch the relationship between changes in catecholamine stimulated contractile activity and cyclic nucleotide metabolism and action was investigated. While septa from aged rats exhibited a diminished contractile response to a maximal dose of isoproterenol, no difference was found either in the elevated levels of cAMP or the increased activation of the cAMP-dependent protein kinase. In addition, no differences were found in the  $K_a$  for cAMP of both the soluble and particulate cAMP-dependent kinases, or the levels of total, soluble, or particulate cAMP-dependent protein kinase. No changes were seen

in total or soluble phosphohistone phosphatase, but particulate fractions from aged septa showed about a 20% increase over that from the septa of young rats. Although other parameters are yet to be evaluated, the cumulative findings, along with the failure of perfusion with dibutyryl cAMP to alleviate the aged difference in contractile response, suggests that the rate-limiting step in aged septa was distal to cAMP generation and protein kinase stimulation.

The relationship between age-associated decrements in striatal dopamine levels plus amphetamine-induced rotational behavior and the activities of tyrosine-hydroxylase, DOPA-decarboxylase, dopamine-stimulated adenylyl cyclase, and cyclic nucleotide phosphodiesterase was examined in collaboration with investigators of the Laboratory of Behavioral Sciences. A 15% decrease in tyrosine hydroxylase was found in senescent rats. The subcellular distribution of the enzyme was not altered. Preliminary results indicate little or no change in DOPA-decarboxylase activity or in the kinetic properties of the enzyme. Results, to date, suggest a decrease in both sensitivity and responsiveness of adenylyl cyclase to dopamine in 24 vs 6 month old rats. No significant age-changes were found in phosphodiesterase activity.

The control of various physiological systems as well as age-related alterations in these vital functions were previously reported to be regulated by hormones, putative neurotransmitters, and drugs whose modes of action were mediated via the cyclic nucleotides, adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP). Thus, studies on the enzymes that determine tissue levels of the cyclic nucleotides, *e.g.* adenylyl cyclase, guanylyl cyclase, and phosphodiesterases, and enzymes, *e.g.* protein kinases, whose activities are regulated by the cyclic nucleotides, are of great significance.

Parathyroid hormone and prostaglandin  $E_1$  receptors and adenylyl cyclase sensitive to these hormones were localized on the basal-lateral membrane of the renal cortical tubule. GMP-PNP, and more effectively, GTP, markedly enhanced the stimulation of adenylyl cyclase by these hormones. For example, parathyroid hormone (20 U/ml) increased adenylyl cyclase 200% relative to the basal activity. With the addition of 0.1  $\mu$ M GTP, which affected only a 10 to 20% activation of the enzyme by itself, parathyroid hormone now stimulated adenylyl cyclase over 400%. The actions of GTP were specific, since the nucleotide did not stimulate the activation of adenylyl cyclase by epinephrine nor glucagon. The presence of cytosolic protein factors which regulated hormonal activation of adenylyl cyclase was also found and these are being isolated and their actions characterized.

Studies on the regulation of phosphate transport were initiated. Two control systems were considered: (1) diet; and (2) regulation by the hormone, parathyroid hormone. The initial rate of phosphate uptake by membrane preparations from animals on a low phosphate diet was about twice that of animals on a high phosphate diet. Preliminary studies suggest that parathyroid hormone, mediated via cyclic nucleotide-dependent systems, altered the uptake of phosphate in membranes isolated subsequent to hormonal treatment.



The permeability properties of membranes are presumed to be regulated by phosphorylation/dephosphorylation of specific membrane proteins, reactions catalyzed by cyclic AMP-dependent and -independent protein kinases and phosphatases. This year we continued to characterize these enzymes in the renal cortex. Protein kinases were localized in both the cytosol and brush border membrane. The cytosol contained two distinct cyclic AMP-dependent and three cyclic AMP-independent kinases, as judged by separation on DEAE-cellulose in cyclic AMP containing buffer. The cyclic AMP-dependent kinases had greater activity with histone F2b as protein substrate than with protamine, whereas the independent forms were more specific for protamine. Salt and detergent extraction of the brush border membrane revealed the presence of two cyclic AMP-dependent and two cyclic AMP-independent enzymes. Kinetic analysis of the cytosolic cyclic AMP-dependent enzymes showed no differences with respect to  $K_a$  for cyclic AMP,  $K_m$  for ATP, or substrate specificity. They did differ with respect to dissociation by salt and histone, and thus were classified as type I and type II (predominantly type II). Both membrane cAMP-dependent enzymes fit the criteria of a type II kinase. Of particular interest was the finding that the membrane protein kinases differed with respect to their ease of extraction from the membrane. High salt concentrations extracted one of the cyclic AMP-dependent and one of the -independent enzymes. The other two kinases, one cyclic AMP-dependent and the other -independent, required detergent for extraction. These findings suggest that the protein kinases are oriented specifically in the membrane, either as intrinsic or extrinsic proteins.

A study was initiated to examine potential regulation of protein kinase activities by a  $Ca^{2+}$ -dependent regulatory protein (calmodulin).

Regulation of the synthesis of cyclic GMP was examined in considerable detail. Noteworthy were the interactions of divalent cations,  $Mn^{2+}$ ,  $Mg^{2+}$ , and  $Ca^{2+}$ , with the cytosolic guanylate cyclase from different tissues. Guanylate cyclase activities of the kidney, liver, and lung were strongly dependent on  $Mn^{2+}$ . In contrast, the enzyme in smooth muscle of the colon, aorta, and vas deferens was active with  $Mg^{2+}$  as well as with  $Mn^{2+}$ .  $Ca^{2+}$  was ineffective in all tissues. Preincubation of colon extracts, but not those of kidney and liver, increased guanylate cyclase activity. The  $Mg^{2+}$ -dependent activity was preferentially enhanced by this treatment. These results suggest that when the enzyme was autoactivated by endogenous factors it became more  $Mg^{2+}$ -dependent. Dithiothreitol strongly inhibited the  $Mg^{2+}$ -dependent colon enzyme, whereas activities in kidney and liver were not affected and the response of the enzyme in lung was intermediate. This suggests that autoactivation involved an oxidative-reductive alteration of the enzyme.  $Ca^{2+}$  markedly inhibited the  $Mg^{2+}$ -dependent activity in smooth muscles but  $Mg^{2+}$ -dependent activities in lung, liver, and kidney were not influenced appreciably. Exogenous activators, dehydroascorbate and  $NaN_3$ , increased guanylate cyclase, assayed with either  $Mg^{2+}$  or  $Mn^{2+}$ . However, the relative stimulation of the enzyme assayed with  $Mg^{2+}$  was greater than with  $Mn^{2+}$ . When activated by these exogenous agents, guanylate cyclase in all tissues became inhibitable by  $Ca^{2+}$ . These findings suggest that guanylate cyclase in smooth muscles was in a partially activated form.



The endogenous activating factors in colon smooth muscle were heat-stable, largely extractable with chloroform:methanol, and cochromatographed with authentic fatty acids. Arachidonic acid stimulated colon guanylate cyclase and enhancement of the  $Mg^{2+}$ -dependent activity was blocked by  $Ca^{2+}$ . This strongly infers that a significant part of the endogenous activating factors in the colon was fatty acids or their derivatives. The colon activators had only minimal effects on the enzyme in the kidney. Similarly prepared activator extracts from the kidney increased colon guanylate cyclase but did not stimulate the renal enzyme. Thus, the ability of the enzyme to be stimulated by endogenous activators was dependent on the tissue from which the enzyme was derived.

These findings strongly support the possibility that the interactions of  $Mg^{2+}$  and  $Ca^{2+}$  on guanylate cyclase in smooth muscles were of regulatory significance to the contractile response of the muscle. It is proposed as a working hypothesis that cyclic GMP and  $Ca^{2+}$  might participate in reciprocal negative feedback mechanisms.

In the course of the above studies on guanylate cyclase in smooth muscle, it was found that colon guanylate cyclase activity was inhibited by the addition of the cytosolic fraction of kidney or liver. This observation suggested that these two latter tissues contained a factor which inhibited the activity of guanylate cyclase. This factor is being purified and its mode of action is being characterized. Preliminary findings indicate that the factor was a protein of approximately 35,000 daltons and that it had the property of binding GTP. However, the capacity to bind GTP and, thus, to remove substrate from the guanylate cyclase reaction was insufficient to account for the inhibition.

Last year we reported that catecholamines were potent activators of cytosolic guanylate cyclase. Studies are continuing on the mechanism of this regulation. Findings to date indicate that the activation was related to the generation of free-radicals during oxidation-reduction of the catechol and the redox state of the enzyme.

The mechanism by which phenothiazines inhibit a partially purified cerebral cortical phosphodiesterase having a high stimulatory response to a  $Ca^{2+}$ -dependent regulatory (CDR) protein and specificity towards guanosine 3',5'-(cyclic) monophosphate was studied. Trifluoperazine (50  $\mu M$ ) completely blocked the  $Ca^{2+}$ -CDR-stimulated activity but had no effect on the basal activity. The magnitude of the inhibition depended on the concentrations of both inhibitor and activator. With increasing concentration of CDR, the enzyme became less sensitive to the phenothiazine. In kinetic analyses of the trifluoperazine-CDR interaction, plots of  $1/V$  vs  $1/CDR$  and  $1/V$  vs trifluoperazine concentration were non-linear with upward curvature. Phenothiazine inhibition was enhanced by decreasing the pH of the reaction mixture from 8.0 to 6.8, a finding which correlated with a previously reported increase in binding of the antipsychotic drug to  $Ca^{2+}$ -CDR when pH was lowered. These results argue against simple competition between inhibitor and  $Ca^{2+}$ -CDR at the activator binding site on the phosphodiesterase. Instead, they suggest that phenothiazines, upon binding to  $Ca^{2+}$ -CDR, render the latter ineffective

in stimulating the enzyme, thus depleting the level of active CDR and inhibiting the activator-dependent phosphodiesterase. It is proposed that analogous phenothiazine- $\text{Ca}^{2+}$ -CDR interactions may be involved in the mode of action of the drug on the activities of other  $\text{Ca}^{2+}$ -CDR-dependent enzymes.

Significance to Biomedical Research and to the Program of the Institute:

These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Studies on mechanisms of hormone regulation will focus on: (1) the mechanism by which  $\text{Ca}^{2+}$  transport in the aged heart sarcoplasmic reticulum can be enhanced, (2) the mechanism of the reduced catecholamine responsiveness in the aged myocardium, (3) the mechanism of the decreased responsiveness of the caudate nucleus of substantia nigra-lesioned aged rats, (4) the characterization and regulation of protein kinase activity, (5) the mechanism for regulation of phosphate transport by diet and hormones, (6) the regulation of guanylate cyclase activity by catecholamines and regulatory protein factors, (7) the regulation of adenylate cyclase activity by hormones, (8) the action of vasoconstrictors and vasodilators on smooth muscle contraction-relaxation and the roles of  $\text{Ca}^{2+}$ , membrane phospholipids, and cyclic nucleotides, and (9) application of these fundamental studies on the hormonal responsiveness in the aged dog.

Publications:

Filburn, C., Colpo, F. and Sacktor, B.: Regulation of cyclic nucleotide phosphodiesterases of cerebral cortex by  $\text{Ca}^{2+}$  and cyclic GMP. J. Neurochem. 30: 337-346, 1978.

Filburn, C. R., Liang, C. T. and Sacktor, B.: Cyclic nucleotide phosphodiesterases of the renal cortex. Characterization of basal-lateral membrane activities. J. Membr. Biol. 37: 29-43, 1977.

Liang, C. T. and Sacktor, B.: The stimulation by catecholamines of guanylate cyclase activity in a cell-free system. J. Cyclic Nucleotide Res. 4: 97-111, 1978.

Sacktor, B.: Regulation of cyclic AMP and cyclic GMP metabolism in renal cortex tubule cells. In Giebisch, G. H. and Purcell, E. F. (Eds.): Renal Function, New York, Josiah Macy, Jr. Foundation, 1978, pp. 290-298.

Sacktor, B., Balakir, R. A. and Filburn, C. R.: Cyclic adenosine 3',5'-monophosphate-dependent and -independent protein kinases of renal brush border membranes. Solubilization, separation and characterization of multiple forms. Arch. Biochem. Biophys. 184: 391-399, 1977.





## Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities. The thrust of the work is focused on mitochondrial metabolism, including regulation of the interaction of carbohydrate and fatty acid oxidative processes and ammoniogenesis and adaptation to acidosis in the aged. This research has impact on the mechanism underlying age-related changes in cardiac and renal function and metabolic homeostasis.

Methods Employed: As model systems, mitochondria isolated from cardiac muscle and kidney are employed.

Major Findings: In the aged, the maintenance of blood pH and the homeostatic mechanism to respond to a state of acidosis by secretion of acid is less competent. Since ammonia production is a major mechanism whereby the kidney produces acid which can be excreted in response to the state of acidosis, a study was initiated to examine the question whether the physiological impairment seen in senescence reflected a biochemical decrement at the level of mitochondrial ammonia genesis and glutamine metabolism. Rats, 6 months and 24 months, were made acidotic by providing 1.5%  $\text{NH}_4\text{Cl}$  solutions as the sole source of drinking water for seven days. It was found that kidney mitochondria from both 6 and 24 month rats showed adaptation to the state of acidosis, with the adaptation being significantly less in mitochondria from the old animals. Thus, values for rates of  $\text{NH}_3$  production, flux through the enzyme glutaminase and flux through glutamate dehydrogenase were  $22.9 \pm 0.9$  ( $21.5 \pm 1.0$ ),  $15.0$  ( $14.5$ ) and  $7.9 \pm 0.2$  ( $7.0 \pm 0.4$ ) nmol/min/mg of mitochondrial protein, respectively, with data from 24 month animals in parentheses. Thus, in the unstressed animals the mitochondria did not differ. In the acidotically stressed animals the corresponding values from 6 month and 24 month old rats were  $38.0 \pm 1.8$  ( $29.6 \pm 2.7$ ),  $23.2$  ( $18.8$ ) and  $14.8 \pm 0.8$  ( $10.8 \pm 1.2$ ). These findings demonstrate that flux through glutaminase and glutamate dehydrogenase, the two reactions responsible for the production of  $\text{NH}_3$  increases significantly in response to acidosis and that the degree of adaptation is significantly less in the old animal. Preliminary observations suggest that far less mitochondrial protein is recovered per kidney or per gram of kidney tissue in 24 month rats relative to 6 month rats and it is considered highly likely that a diminished content of mitochondria in the kidneys of the old animals would be a major contributory factor in limiting  $\text{NH}_3$  production by the kidney in response to acidosis.

Experiments were performed in an attempt to establish the mechanism of the increased flux through glutaminase seen in acidosis. Penetration of the mitochondria by glutamine would limit the flux through this reaction, and was studied directly. Entry of  $^3\text{H}$ -glutamine was characterized with respect to the energetic status of the mitochondrion and the membrane potential and transmembrane pH gradient. A major problem was the metabolism of glutamine to



glutamate within the mitochondrion and this has not been overcome. Attempts were made to use asparagine as a non-metabolizable analogue but there were some doubts that it shared a membrane transport system with glutamine, as asparagine gave no inhibition of  $O_2$ -uptake due to glutamine metabolism.

Isolated kidney mitochondria were also characterized with respect to their ability to oxidize other substrates. Mitochondria from 24 month old rats showed a decreased activity with all substrates tried but the decrease was largest (up to 60%) with acylcarnitine substrates, a result reminiscent of that obtained with heart mitochondria as reported last year.

Work was continued on the interaction between carbohydrate and lipid oxidation, at the level of the pyruvate dehydrogenase. The effect of fatty acids in inhibiting the oxidation of pyruvate by isolated hearts or heart mitochondria was previously reported. Two, mechanistically disparate, forms of control, namely: (1) increased end-product inhibition of the pyruvate dehydrogenase complex by acetyl-CoA and NADH, both known to increase in concentration within the mitochondrion on the introduction of fatty acids; or (2) increased inhibition of pyruvate dehydrogenase by increased phosphorylation of the enzyme protein, a process which leads to inactivation and which is stimulated (at the level of the pyruvate dehydrogenase kinase) by acetyl-CoA and NADH. Studies were aimed to determine the quantitative significance of the two mechanisms.

Mitochondria were incubated with pyruvate in the presence or absence of palmitoylcarnitine and other acyl-group donors and the flux through pyruvate dehydrogenase, measured by the disappearance of pyruvate from the system, was compared with the amount of active (dephosphorylated) pyruvate dehydrogenase found after disrupting the mitochondria and assaying the enzyme under  $V_{max}$  conditions. Then, at a given pyruvate concentration, differences in pyruvate utilization as a proportion of the content of active pyruvate dehydrogenase could be attributed to differences in feed-back inhibition and interpreted in terms of the measured ratios of mitochondrial NAD/NADH and CoA/acetyl-CoA. Experiments of this design showed a generally very great degree of end-product inhibition (as well as enzyme interconversion to the inactive form) in the presence of palmitoylcarnitine as substrate. This is probably the major lipid substrate available to the heart mitochondrion *in vivo*. The predominant mechanism of the end-product inhibition was a decrease in the CoA/acetyl-CoA ratio, owing to the oxidation of the palmitoylcarnitine, though a slightly decreased NAD/NADH ratio was a contributory factor in many of these experiments. An experiment using mixtures of carnitine and acetylcarnitine as substrates, to allow gradual and progressive changes in CoA/acetyl-CoA ratio, gave changes that were in the opposite sense to the changes in NAD/NADH ratio, and thus allowed dissociation of the effects of these two potential regulators. Thus, a decrease in mitochondrial NAD/NADH ratio from 3.5 to 2.2 essentially balanced an increase in CoA/acetyl-CoA ratio from 0.67 to 12 in modulating enzyme interconversion (by phosphorylation), whereas the change in CoA/acetyl-CoA was preponderant in effecting end-product inhibition.

It became clear during this study that the interconversion of pyruvate dehydrogenase by phosphorylation was exquisitely sensitive to the free  $Ca^{2+}$

concentration to which the mitochondria were exposed. Thus decreasing  $pCa^{2+}$  from 6.8 to 6.2, using buffers of  $CaCl_2$  and the chelating agent ethylene glycol bis( $\beta$ -aminoethyl ether)-N,N'-tetraacetate, caused a 10-fold increase in the steady-state content of active pyruvate dehydrogenase, when the inter-conversion system was poised between extremes of the other regulators. Moreover, changes in activity due to changes in  $pCa^{2+}$  were reversible. This is the first time that these effects have been demonstrated in the absence of an ionophore added to make the membrane artificially permeable to  $Ca^{2+}$  and these experiments raise the possibility of pyruvate dehydrogenase regulation by cytosolic  $Ca^{2+}$  concentrations in contractile tissues.

NAD-linked isocitrate dehydrogenase is rate-limiting in the operation of the Krebs cycle in mitochondria. A detailed kinetic analysis was carried out on the effects of various activators and inhibitors of the enzyme, in the presence of divalent cations and chelators. Included in the parameters defined were: (1) the nature of the true substrate; (2) the effects of free divalent cations; (3) the mechanism of  $Ca^{2+}$  inhibition; and (4) the mechanism of ATP inhibition.

Significance to Biomedical Research and to the Program of the Institute:

These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: The oxidation of acylcarnitine compounds was identified as the one mitochondrial activity studied so far which was reproducibly diminished in senescence. The question of whether the transport of acetylcarnitine/carnitine across the mitochondrial membrane is rate-limiting for the metabolism of acetylcarnitine remains open. It is proposed to study this problem further.

There is a discrepancy in the literature concerning the nature of the force driving the accumulation of carnitine across plasma membranes. It is proposed to use renal brush border vesicles as a model membrane system to investigate this problem. Accumulation across the plasma membrane has an important bearing on the carnitine content of non-hepatic tissues and the carnitine content of at least one tissue (heart) has been shown to decrease with age.

It was recently reported that addition of  $CaCl_2$  to isolated liver mitochondria oxidizing fatty acids gave rise to a paradoxical reduction of NAD and an enhanced rate of ketone synthesis. The question of the site of action of the  $Ca^{2+}$  is not known. In view of the longstanding interest of people in this laboratory in both control of mitochondrial oxidations at the dehydrogenase level and in effects of  $Ca^{2+}$  ions, the question of the mechanism of the above effect becomes provocative.

Publications:

Hansford, R. G.: A comparison of energy-yielding reactions in the flight muscle of young adult and senescent blowflies. Comp. Biochem. Physiol. 59B: 37-46, 1978.

Hansford, R. G.: Lipid oxidation by heart mitochondria from young adult and senescent rats. Biochem. J. 170: 285-295, 1978.

Hansford, R. G. and Cohen, L.: Relative importance of pyruvate dehydrogenase interconversion and feed-back inhibition in the effect of fatty acids on pyruvate oxidation by rat heart mitochondria. Arch. Biochem. Biophys., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 AG 00048-04 LMA
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Mechanisms of Metal Ion Transport Across Cellular Membranes		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:  OTHER:	J. P. Froehlich B. Bulos M. Kadoma R. W. Albers  A. S. Hobbs	Medical Officer Research Chemist Visiting Fellow (EOD 3/1/78) Chief, Neurochemistry Section Staff Fellow  LMA NIA LMA NIA LMA NIA LNC NINDCDS  LNC NINDCDS
COOPERATING UNITS (if any) A. Schwartz, Professor and Chairman, Department of Pharmacology, University of Cincinnati; M. Sumida, Postdoctoral Fellow, University of Cincinnati; G. Inesi, Professor, Department of Physiology and Biophysics, University of the Pacific		
LAB/BRANCH Gerontology Research Center, Laboratory of Molecular Aging		
SECTION Biophysics and Intermediary Metabolism Sections		
INSTITUTE AND LOCATION NIA, NIH, Baltimore, Maryland 21224		
TOTAL MANYEARS:  1.4	PROFESSIONAL:  1.2	OTHER:  0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) (1) <u>Rapid reaction techniques</u> were used to distinguish the kinetic effects that arise from preincubation with $\text{Na}^+$ and $\text{K}^+$ on the partial reactions catalysed by <u><math>(\text{Na}^+ + \text{K}^+)\text{ATPase}</math></u> . Results from brief exposure to $\text{K}^+$ in the absence of $\text{Na}^+$ or prolonged exposure to $\text{K}^+$ in the presence of $\text{Na}^+$ suggest that $\text{Na}^+$ and $\text{K}^+$ binding sites could be simultaneously occupied with the implication that these species might be cotransported. (2) The <u>age-dependent deficit</u> in the <u>inotropic response</u> of the myocardium to <u>ouabain</u> was found to be at a site distal to the binding of the cardiac glycoside to the <u><math>(\text{Na}^+ + \text{K}^+)\text{ATPase}</math> receptor</u> . (3) Measurement of the <u>rapid kinetics</u> of <u>Ca ATPase</u> in <u>cardiac</u> and <u>skeletal muscle sarcoplasmic reticulum</u> suggest an explanation for the <u>slower relaxation rate</u> of the <u>cardiac muscle</u> . (4) Studies of the regulation of <u>Ca uptake</u> by sarcoplasmic reticulum from young and <u>aged animals</u> were continued.		

GRC/LMA-232



## Project Description:

Objectives: (1) To provide a model for active cation transport by elucidation of the enzymatic pathways linked to cation transport; (2) to define the molecular basis of age-dependent changes in active transport systems involved in the regulation of muscular contraction and relaxation.

Methods Employed: Measurements of  $^{45}\text{Ca}$  accumulation by sarcoplasmic reticulum membranes isolated from cardiac muscle homogenates and treated with cAMP and protein kinase were carried out by Millipore filtration. The effects of a brief exposure to  $\text{Na}^+$  or  $\text{K}^+$  on the kinetics of the partial reactions catalysed by  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  were investigated with the aid of a rapid mixing apparatus that enables two successive mixing operations to be carried out before the addition of the stopping reagent.

Major Findings: The hydrolysis of ATP by  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  was shown to occur by a mechanism that involves  $\text{Na}^+$ -dependent acylphosphoenzyme formation followed by  $\text{K}^+$ -dependent cleavage of the phosphoenzyme bond. These reactions are believed to occur consecutively such that  $\text{Na}^+$  was bound to and released from the enzyme before the interaction with  $\text{K}^+$  took place. The early time course of phosphorylation and  $\text{P}_i$  release in response to the addition of ATP following preincubation with  $\text{Na}^+$  and/or  $\text{K}^+$  was examined and found that  $\text{K}^+$  in the presence of  $\text{Na}^+$  increased the rate of dephosphorylation compared to the case in which only  $\text{Na}^+$  was present in the preincubation medium and ATP hydrolysis was initiated by addition of ATP plus  $\text{K}^+$ . Brief (20 msec) exposure to  $\text{K}^+$  in the absence of  $\text{Na}^+$  also activated the rate of dephosphorylation, whereas prolonged exposure to  $\text{K}^+$  led to inhibition of phosphorylation and  $\text{P}_i$  release upon addition of ATP plus  $\text{Na}^+$ . These experiments demonstrate that  $\text{K}^+$  bound prior to phosphorylation could activate dephosphorylation and thus suggest that  $\text{Na}^+$  and  $\text{K}^+$  binding sites were occupied simultaneously rather than consecutively.

The early time course of ATP hydrolysis measured by the acid quench technique showed a transient phase of product liberation ( $\text{P}_i$  burst) that is believed to represent the formation of an acid-labile phosphorylated intermediate. At neutral pH, hydrolytic cleavage of the intermediate yielded free phosphate and a proton at rates commensurate with the overall velocity of ATP hydrolysis. To test whether phosphate appearing during the burst phase was bound to the enzyme or released, stopped-flow measurements of the transient phase of ATP hydrolysis were carried out at neutral pH using phenol red as proton indicator. The experiments showed that proton liberation lagged  $\text{P}_i$  production measured during the burst phase indicating that the  $\text{P}_i$  was covalently bound to the enzyme prior to the addition of acid.

In collaboration with investigators from the Clinical Physiology Branch, the inotropic response to ouabain was observed to be diminished in the myocardium of aged (25 months) relative to adult (6 months) rats. It was found that ouabain-sensitive ATPase ( $\text{Na}^+\text{K}^+\text{ATPase}$ ) in the rat heart, expressed as a percent of the total ATPase activity of adult and aged animals, was not significantly different,  $27.1 \pm 5.4$  for the young and  $24.6 \pm 5.6$  for the

senescent heart. The ouabain concentration dependence of the inhibition of Na<sup>+</sup>K ATPase was also the same in the two age groups. Thus, the decrement in inotropic response to ouabain seen in the aged myocardium, which occurred over the same concentration range that enzyme inhibition was found, suggests that the age-dependent deficit was at a site distal to the binding of the cardiac glycoside to the Na<sup>+</sup>K ATPase receptor.

Current evidence supported similar functions and mechanisms for cardiac sarcoplasmic reticulum (CSR) as for skeletal SR (SSR). It was thought that the slower relaxation rate of cardiac muscle compared to fast skeletal muscle reflected the lower ATPase activity and calcium transport of CSR. Possible qualitative differences in the isolated preparations were studied by using a quench flow apparatus capable of measuring phosphorylation, dephosphorylation and calcium transport with accuracy and precision. The results showed that both CSR and SSR bound calcium tightly in the absence of ATP, and coupling of E<sub>v</sub>P formation and calcium transport occurred in the transient phase of ATP hydrolysis. The rate of phosphorylation ( $t_{-1/2}$  10 msec) of SR preloaded with calcium was the same for cardiac and skeletal preparations. However, the rates of dissociation of extravesicular calcium (9 sec<sup>-1</sup> vs 15 sec<sup>-1</sup>), phosphorylation of calcium-free SR, and dephosphorylation of E<sub>v</sub>P (8 sec<sup>-1</sup> vs 12 sec<sup>-1</sup>) were lower for cardiac than for skeletal SR. By analog simulation, the apparent rate constants associated with the reduced rates of phosphorylation of calcium-free SR were: 12 sec<sup>-1</sup> for cardiac and 63 sec<sup>-1</sup> for SSR. The difference in the rates might be partly responsible for slower turnover of the phosphorylated intermediate and the lower levels of calcium transport activity which characterize cardiac muscle preparations.

Studies were initiated, using the techniques of membrane reconstitution, to determine whether the decrement in Ca<sup>2+</sup> uptake in the sarcoplasmic reticulum from hearts of the aged was due to aged-dependent alterations in the pump protein or in the membrane milieu. Because of increased yield and purer membrane preparations, the sarcoplasmic reticulum of skeletal muscle was employed as a model system. The kinetic parameters of Ca<sup>2+</sup> uptake by this preparation is being characterized.

#### Significance to Bio-medical Research and to the Program of the Institute:

The alterations in cardiac mechanical performance associated with aging ultimately relate to changes in the enzymatic and structural properties of the proteins that comprise the contractile machinery and transport systems involved in excitation-contraction coupling. Information provided by these studies help to promote a clearer understanding of the causes underlying altered mechanical performance and thus shed light on the mechanism(s) responsible for the progressive decline in physiologic activity that is part of the aging phenomenon.

Proposed Course of the Project: The program aimed at characterizing the properties of SR prepared from old hearts will be broadened to include (1) an examination of Ca<sup>2+</sup> accumulation in the transient state, and (2) an investigation of the kinetics of passive and active Ca<sup>2+</sup> efflux to determine the significance of these fluxes to the diminished Ca<sup>2+</sup> transport rates. Rapid

mixing studies of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  and  $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$  will be continued with emphasis placed on the properties of the reactions between these enzymes and their ligands. Studies to reconstitute the  $\text{Ca}^{2+}$  uptake function of synthetic SR membranes will be continued to determine whether the decrement in  $\text{Ca}^{2+}$  uptake in the aged heart represents change in the pump protein or the membrane.

Publications:

Chou, H. J., Froehlich, J. P. and Pitha, J.: Template specific inhibitors of *E. coli* RNA polymerase. Nucleic Acids Res. 5: 691-696, 1978.

Froehlich, J. P., Lakatta, E. G., Beard, E., Spurgeon, H. A., Weisfeldt, M. L. and Gerstenblith, G.: Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. J. Mol. Cell. Cardiol. 10: 427-438, 1978.

Sumida, M., Schwartz, A. and Froehlich, J. P.: Preliminary observations of presteady state kinetics of phosphorylation and dephosphorylation of sarcoplasmic reticulum membranes from heart and skeletal muscle. Ann. N. Y. Acad. Sci. 307: 228, 1978.

Sumida, M., Wang, T., Mandel, F., Froehlich, J. P. and Schwartz, A.: Transient kinetics of  $\text{Ca}^{2+}$ -transport of sarcoplasmic reticulum: a comparison of cardiac and skeletal muscle. J. Biol. Chem., in press.



Annual Report of the Laboratory of Neurosciences  
National Institute on Aging

The Laboratory of Neurosciences at the National Institute on Aging was formed in 1978, to conduct research on the central and peripheral nervous systems and muscle in health, disease and aging. At the present time, two senior investigators are in the laboratory, myself, and Dr. C. C. Chiueh, a Senior Staff Fellow. My interests have been the blood-brain barrier and muscle fatigue, as well as more general questions concerning regulation of nervous system function and pharmacology of the nervous system. Dr. Chiueh is a neuropharmacologist who has worked on stress, hypertension, and psychoactive drugs in relation to sympathetic-adrenal function.

These annual reports are presented as three projects: (A) Blood-brain barrier function and central nervous system function, (B) function of peripheral nerve and muscle, and (C) pharmacology of the central and peripheral catecholaminergic nervous system. I am primarily responsible for the first two projects, whereas Dr. Chiueh is responsible for the third.

Studies of blood-brain function in health and disease require quantitative measurements of cerebrovascular permeation. Techniques for measuring vascular permeability generally are insensitive, semi-quantitative and influenced by changes in cerebral blood flow. We therefore elaborated, experimentally and theoretically, a quantitative procedure that is independent of cerebral blood flow and at least 1000 times more sensitive than any currently available.

We used this method to measure  $^{14}\text{C}$ -sucrose permeability at cerebral blood vessels of mature and senescent Fisher 344 rats. Sucrose enters the brain very slowly, and a slight alteration of cerebrovascular integrity would easily be noticed as an increase in permeability. We found, however, that  $^{14}\text{C}$ -sucrose permeability was not changed in senescent rats. Thus, we could not support the hypothesis (at least for the rat) that aging of the brain is caused by entry of blood-borne, brain-reactive antibodies across a defective blood-brain barrier.

A major problem in psychopharmacology is that a drug response often cannot be related directly to drug dosage or plasma concentration. In the elderly, furthermore, the high incidence of neurotoxicity may be caused by a high brain concentration of a drug at a dosage that is not toxic in young individuals, or by altered receptor sensitivity to a drug. Using our method as discussed above, we established a general relation between cerebrovascular permeability and the octanol/water partition coefficient of a drug. We also formulated a multi-compartment (brain-plasma) kinetic model that incorporates the observed relation between permeability and partition coefficient, and that can be used to predict brain concentration of a psychoactive drug from the dosage and plasma concentration.

In 1972, we first demonstrated that the blood brain barrier could be reversibly opened in animals by infusing a hypertonic solution of a water-soluble nonelectrolyte into the carotid artery. The effect later was shown



to be caused by osmotic shrinkage of cerebrovascular endothelial cells, with consequent widening of interendothelial tight junctions. In the last year, we experimentally refined the osmotic method in the rat, and quantitated effects of such parameters as infusate concentration and infusion time on cerebrovascular permeability. We showed for the first time that when the barrier opens transiently brain metabolism is stimulated and a temporary brain edema is produced, and thus that barrier integrity must be maintained continuously for normal brain function. We also employed the osmotic method as a pharmacological tool, in the study of cerebral effects of vasoactive amines, exogenous enzymes and blood-borne antibodies.

Contrary to the generally accepted notion that muscle fatigue is due to exhaustion of available energy in the form of ATP, we showed that single frog muscle fibers became fatigued in response to tetanic stimulation when ATP concentrations remained normal. Intracellular ATP was not sequestered, furthermore. Our findings indicate that muscle fatigue can arise from failure of excitation-contraction coupling rather than from energy exhaustion. Accumulated  $H^+$  (as lactic acid) may promote this failure. It is known that fatigability can be reduced in the young as well as in the elderly by endurance training, which increases the mitochondrial content and capacity for aerobic metabolism of muscle. If our hypothesis is correct, that  $H^+$  (as lactate) accumulation can result in fatigue, then reduced fatigability in endurance-trained muscles could be related to their increased capacity to oxidize lactic acid.

Dr. Chiueh demonstrated that the accepted mechanism for the central and peripheral action of cocaine is not correct. Cocaine generally is assumed to act on the nervous system primarily by inhibiting reuptake of catecholamines that are released from nerve endings during activity. However, Dr. Chiueh showed that cocaine increases the release of catecholamines from both central and peripheral aminergic systems by augmenting neuronal firing through a local anesthetic effect on inhibitory neurons. On the other hand, amphetamine acts directly at nerve terminals to induce catecholamine release.

Dr. Chiueh also developed a free-running rat model to study the effects of stress on catecholamine release. He showed that resting levels of plasma catecholamines in conscious undisturbed animals were less than 0.5 ng/ml, but were increased substantially by decapitation, immobilization and other forms of stress. He intends to use the free-running model to study the activity of the peripheral sympathetic nervous system under various physiological conditions.

Dr. Chiueh also discovered that current methods which use acidic metabolites of catecholamines as indices of catecholamine turnover in the nervous system are incorrect. He consequently developed a mass-spectrometric assay procedure for O-methylated catecholamines that provides correct measurement of turnover. He intends to employ this procedure to study catecholamine turnover in relation to stress, hypertension, malnutrition and aging.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00120-01 LNS (Formerly Z01 MH 01083-12 LNP)

PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Blood-Barrier and Central Nervous System Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.I. Rapoport	Chief	LNS NIA
	C.C. Chiueh	Sr Staff Fellow	LNS NIA
	K. Ohno	Visiting Fellow	LNP NIMH
Others:	W.K. Fredericks	Technician	LNS NIA
	L. Sokoloff	Chief	LCM NIMH
	J.A. Barranger	Neurologist	DMN NINCDS
	R.O. Brady	Chief	DMN NINCDS
	K.D. Pettigrew	Statistician	B NIMH

COOPERATING UNITS (if any) Laboratory of Neurophysiology, NIMH  
Laboratory of Cerebral Metabolism, NIMH  
Developmental and Metabolic Neurology Branch, NINCDS  
Division of Biometry and Epidemiology, NIMH

LAB/BRANCH

Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYLANS:

2.7

PROFESSIONAL:

1.7

OTHER:

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacokinetic principles for the central nervous system were established by demonstrating in rats that cerebrovascular permeability of nonelectrolyte and organic electrolytes is related linearly to the octanol/water partition coefficient. Drug entry into the brain now can be predicted from the partition coefficient and plasma concentration. Permeability to <sup>14</sup>C-sucrose is very low and unaffected by senescence in the rat. This suggests, contrary to the immune hypothesis for brain aging, that the blood-brain barrier at the cerebral vasculature is not disrupted in the aged brain. Barrier permeability can be reversibly increased by infusing a hypertonic solution of arabinose into the carotid artery of rats. Tight junctions between cerebrovascular endothelial cells widen to intravascular substances. The osmotic method to open the blood-brain barrier transiently elevates brain water and regional brain glucose consumption, but does not produce long-term cerebral pathology. It has been used as a pharmacological tool in animals to allow protein antibodies and enzymes into the brain, as well as amines which modify cerebral blood flow.

Objectives: The blood-brain barrier at the cerebral vasculature regulates the ionic environment of the nervous system, has specific mechanisms for transport of amino acids and glucose that support cerebral metabolism and neurotransmitter and protein synthesis, and prevents access to the brain of water-soluble drugs and other agents. The aims of this project are as follows: (a) to describe the ultrastructure of the blood-brain barrier in relation to its permeability and physiologic function in normal and aging brain, (b) to determine the quantitative rules that govern drug entry into the brain from plasma, (c) to develop a method to reversibly modify barrier permeability, and to apply it to understanding the central actions of systemically-administered drugs and other agents, and (d) to determine how the barrier regulates behavior and brain metabolism.

Methods Employed: A number of different methods were employed in these studies. Surgical procedures were used to open the skull and dura of anesthetized animals, and to catheterize blood vessels for infusion and plasma sampling. Neurological examinations and behavioral tests evaluated overall brain function and localized deficits. Histological methods were used to examine brain pathology, and electronmicroscopy was employed to evaluate transfer mechanisms at the barrier and barrier ultrastructure. Radioisotope techniques including autoradiography were used to quantitate drug and metabolite transfer from blood to brain. Computers were used to analyze data and to generate relevant models.

Major Findings: A. Quantitative Aspects of Drug Entry into Central Nervous System.

1. Central nervous system pharmacokinetics. The blood-brain barrier limits exchange of drugs between plasma and brain, and makes it difficult to interpret dose-response relations of centrally-acting drugs. We therefore decided to establish empirical rules to quantitatively predict drug entry into the brain from the plasma concentration and the physical properties of the drug (e.g., lipid solubility). Radiotracers of organic electrolytes and of non-electrolytes, with differing octanol/water partition coefficients, were injected intravenously in conscious rats. Arterial plasma concentrations were followed, and regional brain concentrations of tracer were determined at various times after injection. A model for blood-brain exchange was formulated and applied to the data. Calculated cerebrovascular permeability was found to be related linearly to the octanol/water partition coefficient of a solute. For a drug whose partition coefficient is known, the rate of brain accumulation can be predicted now from the linear relation and the history of the plasma concentration. Cerebral blood flow, which was measured in a separate series of experiments with  $^{14}\text{C}$ -iodoantipyrine, must also be known for predicting brain accumulation of very permeable drugs.

The method for measuring permeability and predicting drug entry into the brain is at least 1000 times more sensitive than any currently available (indicator dilution technique or brain uptake index technique), and should be applicable to pharmacokinetic studies in man. An abstract of this work has been published: K. Ohno, K.D. Pettigrew and S.I. Rapoport. Cerebrovascular



permeability to water-soluble nonelectrolytes. Biophysical Journal 17 (No.2), 173a, 1978.

2. Blood-brain barrier integrity in senescent rats. A proposed hypothesis for aging of the central nervous system is that the blood-brain barrier breaks down so as to allow potentially neurotoxic brain-reactive antibodies in plasma into brain. We tested this hypothesis in rats by measuring cerebrovascular permeability to  $^{14}\text{C}$ -sucrose (see above) in 3-month and 28-month conscious Fisher 344 rats. Sucrose permeability was equal in both groups. As it normally is extremely low,  $2 \times 10^{-8}$  cm/sec, any change in permeability would be easily measured. The findings thus do not support the immune hypothesis for brain aging. We did find, however, that the brain extracellular space was reduced by about 50% in aged animals, in accord with Bondareff's findings with freeze-substitution. A reduced space might limit intercellular diffusion within the aged brain. An abstract of this work has been published: S.I. Rapoport, K. Ohno and K.D. Pettigrew. Blood-brain barrier in aged rats. Abstr. 8th Annual Meeting Neuroscience Society, 1978 (in press).

## B. Reversible Modification of Blood-Brain Barrier Permeability.

1. Methods and mechanism. The blood-brain barrier at cerebral capillaries is due to a continuous layer of endothelial cells that are connected by tight junctions. We demonstrated that the barrier can be opened reversibly by infusing a hypertonic solution of a water-soluble solute (e.g., arabinose) into the internal carotid artery of rats, rabbits or monkeys. We also showed, with electronmicroscopy and by theoretical models for osmotic action that barrier opening probably is caused by shrinkage of cerebrovascular endothelial cells and widening of interendothelial tight junctions. The method should prove useful as a tool for studying central actions of drugs that normally do not penetrate the blood-brain barrier (Refs. 3 and 4).

Osmotic barrier opening can be accomplished in animals without producing long-term changes in behavior or brain water and electrolytes (Ref. 1). However, if the procedure is employed in conscious rats, then transient changes occur that are reversed within 2 to 24 hr. Brain water is elevated by about 1%, due to barrier opening to salts and loss of the protective barrier action against brain edema. Cerebral blood flow no longer is tightly coupled to cerebral metabolism. Finally, behavioral and cerebral metabolic changes reminiscent of convulsions (some changes are prevented by valium) are produced by barrier opening. Regional uptake is increased of  $^{14}\text{C}$ -2-deoxy-D-glucose. This tracer is a glucose analogue that is transported and phosphorylated like glucose within the brain but not metabolized further. An abstract of some of this work has been published: H.M. Pappius, H. Savaki, C. Fieschi, S.I. Rapoport and L. Sokoloff. Cerebral glucose utilization after opening of blood-brain barrier. Trans. Amer. Society Neurochemistry 9 (1), 116, 1978.

2. Pharmacological uses. Osmotic barrier opening has been employed by us to allow the following normally-excluded agents into the brain:

a. Neutralizing antibody (IgG) to measles virus. In the rhesus monkey that is immunized to measles, neutralizing antibody is increased in the brain



following osmotic barrier opening by hypertonic arabinose solution. Antibody remains in the brain for up to 4 days (Ref. 5).

b. Brain enzyme  $\alpha$ -mannosidase. The purified enzyme was infused intravenously in rats following osmotic barrier opening by hypertonic mannitol or arabinose solution. Enzymatic activity increased from a mean of 1250 units/g wet wt in control brains to 2467 units/g wet wt in infused brains. The increase was equivalent to an amount of enzyme that would be required to restore  $\alpha$ -mannosidase activity to normal levels in a totally deficient animal. The brain, normally inaccessible to systemically administered enzymes, can now be considered a potential target organ for enzyme replacement therapy. This work is published in abstract form: J.A. Barranger, P.G. Pentchev, S.I. Rapoport, R.O. Brady. Augmentation of brain lysosomal activity following enzyme infusion with concomitant alteration of the blood-brain barrier. Annals Neurology 1, 496, 1977.

c. Norepinephrine. The osmotic method has been employed to study the central action of amines and other agents on cerebral blood flow. We measured the rate of entry of norepinephrine and its metabolites into brains of rats administered  $^3\text{H}$ -norepinephrine intravenously, with and without osmotic opening, to determine thresholds for central effects on blood flow. We also showed that tracer was taken up at a finite rate by the normal brain contrary to previous reports (Ref. 2).

#### Significance to Biomedical Research and to the Program of the Institute:

1. An understanding of how the blood-brain barrier regulates the brain environment and controls brain metabolism is critical to interpreting changes in brain function in health, aging and disease. Our findings establish the ultrastructural and transport properties of the blood-brain barrier.
2. Reversible osmotic barrier opening is the first and only method that allows normally excluded agents into the brain without producing long-term brain damage. Our experiments with protein antibodies and enzymes suggest that the method can be employed for enzyme replacement therapy, for studying interaction between brain tissue and the peripheral immune system, or for studying effects of centrally-potent drugs on brain metabolism or blood flow.
3. The finding that reversible barrier opening transiently increases regional brain metabolism and brain water content shows for the first time why barrier integrity must be maintained for normal cerebral function. Some effects of trauma, hypertension and infection might be due indirectly to barrier opening.
4. Our method to measure cerebrovascular permeability to drugs will make it possible to predict and interpret dose-response relations of centrally acting drugs in man and animals. An altered response with age could be due to an altered rate of drug entry into the brain or to changes in cerebral receptor sensitivity.

5. As cerebrovascular integrity is not increased in aged rats, the hypothesis that entry of brain-reactive proteins into brain augments cell death must be reevaluated. The calculated decline in brain extracellular space with age suggests that intercellular diffusion is altered in the senile brain.

Proposed Course: Work previously done under Project Number Z01 MH 01083-12 LNP, "Transport Mechanism Activity Membrane Under Blood-Brain Barrier."

Project to be continued: (a) The pharmacokinetic rules governing drug transfer from plasma to brain will be elaborated, and models will be developed to predict and interpret dose-response relations of centrally-acting drugs in animals and man, (b) transport of substrates for brain metabolism will be quantitated at the cerebral vasculature, in relation to malnutrition and aging, (c) further ultrastructural studies will be made to define the basis of increased cerebrovascular permeability following osmotic treatment, (d) the time course of osmotic opening of the blood-brain barrier will be specifically related to infusion time and infusate concentration, so as to apply the osmotic method to drug entry into the brain, (e) the genesis and resolution of cerebral edema will be evaluated in terms of transient and long-term opening of the blood-brain barrier, and (f) sites and mechanisms for protein entry into cerebrospinal fluid will be analyzed.

#### Publications:

Rapoport, S.I., Matthews, K., Thompson, H.K. and Pettigrew, K.D.: Osmotic opening of the blood-brain barrier in the rhesus monkey without measurable brain edema. Brain Research 136: 23-29, 1977.

Chiueh, C.C., Sun, C.L., Kopin, I.J., Fredericks, W.R. and Rapoport, S.I.: Entry of  $^3\text{H}$ -norepinephrine,  $^{125}\text{I}$ -albumin and Evans blue from blood into brain following unilateral osmotic opening of the blood-brain barrier. Brain Research 145: 291-301, 1978.

Rapoport, S.I.: Osmotic Opening of the Blood-Brain Barrier. In M.J. Purves, (Ed.): Ciba Foundation Symposium No. 56 (new series.) Cerebral Vascular Smooth Muscle and its Control. Amsterdam, Elsevier, 1978, pp. 237-255.

Rapoport, S.I.: Osmotic Opening of Blood-Brain and Blood-Ocular Barriers. In Bito, L.Z., Davson, H., Fenstermacher, J.D. (Eds.): The Ocular and Cerebrospinal Fluids. (Proceedings of a Fogarty International Center Symposium, Bethesda, Md. 3-6 May 1976). Experimental Eye Research 25 (Supplement): 499-509, 1977.

Rapoport, S.I.: Reversible Modification of Blood-Brain Barrier to Proteins. In Nandy, K., Sherwin, I. (Eds.): The Aging Brain and Senile Dementia. New York, Plenum Press. Advances in Behavioral Biology 23: 197-208, 1977.

Pentchev P.G., J.W. Kusiak, J.A. Barranger, F.S. Furbish, S.I. Rapoport, J.M. Massey and R.O. Brady. Factors that Influence the Uptake and Turnover of Glucocerebrosidase and  $\alpha$ -Galactosidase in Mammalian Tissues. In Gatt, S., Freysz, L., and Mandel, P. (Eds.): Enzymes of Lipid Metabolism. New York, Plenum Press, 1978, pp. 745-752.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00121-01 LNS (Formerly Z01 MH 01082-13 LNP)																																			
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SUMMARY OF WORK (200 words or less - underline keywords) <p>           A. <u>Fatigue of single frog muscle fibers</u> appeared as a decline in tension output during prolonged <u>tetanic stimulation</u>. The concentration of <u>ATP</u> (energy source for <u>contraction</u>) remained normal in fatigued fibers. Furthermore, ATP was not sequestered or unavailable for contraction. Fatigue therefore was due to an inability of the muscle to use energy in the form of ATP. We suggest that <math>H^+</math>, as <u>lactic acid</u>, accumulated in these fibers so as to interfere with <u>excitation-contraction coupling</u>. B. The <math>^{14}C</math>-2-deoxy-D-glucose method, which was developed to measure regional cerebral metabolism, was extended in this study to analyze <u>regional metabolism</u> in the quadriceps femoris <u>muscle</u> of the rat. Stimulation of the femoral nerve at different frequencies produced different patterns of increased metabolism. The basis for these patterns remains to be determined. C. The <u>perineurial sheath</u> which surrounds <u>peripheral nerve axons</u> may regulate nerve function and development. Perineurial <u>permeability</u> was measured for the firsttime (to <math>^{14}C</math>-sucrose) and was found to be very low, supporting a <u>regulatory role</u> for the sheath. Permeability was increased by stretch and by soaking in hypertonic solutions.         </p>																																					

GRC/LNS-243



Project Description:

The object of this project is to relate metabolism and morphology of peripheral nerve and muscle to the functioning of these tissues in health, disease and aging.

Objectives.

A. Muscle contraction and muscle fatigue. When a striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue characterizes fast twitch muscles whose metabolism is glycolytic, rather than slow twitch muscles whose metabolism is oxidative. Muscle fatigue generally is thought to be caused by depletion of available energy reserves. However, some studies in single fibers suggest that fatigue can be due to uncoupling of steps that connect excitation with contraction. We explored the relation between fatigue and metabolism in single muscle fibers rather than in whole muscles, because, in whole muscles, some fibers can fatigue faster than others and measured metabolite concentrations are averaged over a heterogeneous, often disparate fiber population. Furthermore, intercellular diffusion limits physiological studies of whole muscles but not of single fibers.

B. Regional muscle metabolism. The above studies showed that single fibers are better for studying metabolism-function relations in muscle than are whole muscles with mixed fiber populations. We also wanted to see if local rather than regional metabolism could be measured in whole muscle in relation to function. We employed the  $^{14}\text{C}$ -2-deoxy-D-glucose (DG) method (Sokoloff et al., J. Neurochem. 28, 897, 1977) for this study. Like glucose, for which DG is an analogue, DG is phosphorylated in muscle and brain to DG-6-P. As glucose-6-phosphatase (the enzyme most likely to hydrolyze DG-6-P) is low in muscle, radioactivity is not rapidly lost and DG-6-P accumulation may be proportional to the rate of regional glucose consumption.

C. Peripheral nerve and perineurium. Peripheral nerve function in health and disease may be governed in part by the local axonal milieu, which is separated from body fluids by a perineurial sheath and by endoneurial capillaries. We decided to examine the perineurium, whole role in regulating the axonal environment is not known. The sheath is a multilayered structure, one layer of which acts as a diffusion barrier because it is composed of cells that are closely connected by tight junctions (zonulae occludentes).

Methods Employed:

A. A single fiber was dissected from the frog semitendinosus muscle and mounted in a bath of flowing Ringers solution at  $15^{\circ}\text{C}$ . Tension was recorded by a RCA 5734 transducer tied to one end of the fiber; the output was displayed on an oscilloscope face. Sarcomere length was measured by a laser diffraction technique. The fiber was stimulated at different frequencies via external platinum electrodes, so as to produce twitches or tetanic



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